CHAPTER 2

Review of the literature

2.1 Taxa and Classification of *Graptophyllum pictum* (L.) Griff. (20)

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteridae

Order: Scorphulariales

Family: Acanthaceae (Acanthus family)

Genus: *Graptophyllum* Nees. (graptophyllum)

Species: *Graptophyllum pictum* (L.) Griff.

Synonyms (21)**:** *Justicia picta*, *Graptophyllum hortense*.

Common names (21-22)**:** Caricature-plant (English), Lait de la Vierge (French), Graptophylle peint (French), Benalu (Malay), Daun ungu (Indonesian), Bai Ngeon (Thai), Morado (Filipino).

Figure 2.1 *Graptophyllum pictum* (L.) Griff.

2.2 Review of traditional use, chemical composition and bioactivities of *Graptophyllum pictum* (L.) Griff.

Graptophyllum pictum (L.) Griff. is a Papua New Guinea native shrub (Figure 2.1). It is a shrub with a straight stem. Its leaf is simple, opposite, ovate, glossy green and variegated with pale yellow or white marks on the leaf. The flower is purple or red terminal inflorescences which has dark red, two-lipped and tubular corolla. The fruit is elliptic capsules. Although, *G. pictum* is often cultivated in the tropics as an ornamental plant, it is still used as a traditional medicine (21,23-24).

In New Guinea, *G. pictum* flowers have been used to make tea (23). In Indonesia and Malaga Island, the poultice from the leaf has been used to relieve swollen pain and abscess and a tea made from the flower has been used to stimulate menstrual flow. In Malaysia and Philippines, they use *G. pictum* leaf to stop bleeding, heal cuts, wounds, ulcers and to assuage swollen parts. *G. pictum* juice has been used to relieve earache, skin diseases and anti-constipation, while the leaf decoction is used to soothe the bowels of costiveness, heal hemorrhoids and expel gallstone (23,25). In India, the leaf is used for breast inflammation, scorpion sting and is used as an emollient (26). In a Sudanese village, the decocting, heating or pounding of *G. pictum* leaf was used to treat fever and

postpartum remedy (27). In Thailand, *G. pictum* root has been used as an antidote, a detoxifying agent and an expectorant. Its stem has been used as an antipyretic and to heal an aphthous ulcer (28). The leaf has been used as a diuretic, an antipyretic, an anti-constipation, an anthelmintic, to heal aphthous ulcer, sooth earache and swollen parts. Its flower has been used to stimulate menstrual flow. Pollen has been also used as an anti-fatigue and an antipyretic (25,28-29).

Previous phytochemical analyses of the *G. pictum* extract indicated vomifoliol (Figure 2.2) (30) and flavonoids (31).

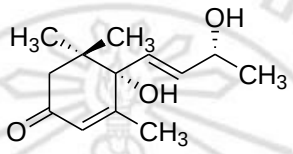


Figure 2.2 The structure of vomifoliol.

The biological activities of the plant extracts have been reported, the ethanolic extract showed anti-inflammatory activity (31), reduced cisplatin-induced nephrotoxicity (32) and it also showed oxytocic and anti-implantation activities which can be used as a contraceptive in very early pregnancy (33). The aqueous extract of the leaves reduced blood glucose levels (34), while the *n*-butanol and water fractions showed alkaline phosphatase (ALP) stimulatory activity against MC3T3-E1 osteoblast cells. The ALP activity is a marker of osteoblast differentiation which involves in bone formation (35). Another study showed that the dichloromethane extract of the aerial parts of *G. pictum* decreased the growth of bacteria (*Xanthomonas campestris*) and slightly inhibited fungi; *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Phytophthora parasitica*, whereas the methanolic extract inhibited only the growth of *Sclerotium rolfsii* (36).

2.3 Taxa and Classification of *Solanum spirale* Roxb. (37)

Kingdom: Plantae (Plants)

Subkingdom: Viridaeplantae (Green plants)

Infrakingdom: Streptophyta (Land plants)

Division: Tracheophyta (Vascular plants)

Subdivision: Spermatophytina (Seed Plants)

Infradivision: Angiospermae (Flowering plants)

Class: Magnoliopsida

Superorder: Asteranae

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species: *Solanum spirale* Roxb.

Synonyms (38-39): *Solanum apoense* Elmer, *Solanum callium* R.J.F. Henderson, *Solanum naratida* Buch.-Ham. Ex well, *Solanum spirale* var. tetrasepalum H.J. Chu., *Solanum superficiens* Adelb.

Common names (39-41)**:** pak dit, pak dip, puk dip, ping pang, yadeedfai (Thailand); pak dit, mak dit (Lao); xuan hua qie (Pinyin, China); titikuchi (Assam), oko-oing (Miri), lara-tita (Goalpara), sohjaring, soh-jhari (Khasia), khlein-deng (Synt.) India.

Figure 2.3 *Solanum spirale* Roxb.

2.4 Review of traditional use, chemical composition and bioactivities of *Solanum spirale* Roxb.

Solanum spirale Roxb. is a small shrub with a 0.5-4 m height of erect stem (Figure 2.3). The young stem is green and turns to dark brown when it is old. The leaves are simple, lanceolate, opposite, serrated-edge and glabrous above. The major leaves are narrowly elliptic to elliptic, 9-22 cm long and 4-11 cm wide, while the minor leaves are the same style except in size, 5-7 cm long and 1.6-3 cm wide. The inflorescences are opposite the leaves, 2-8 cm long, 5-30 flowers, round pedicel in 1.5-3.0 cm long. The flower had 5 lobes of white apices petals which arrange in 5-angled and the 5 lobes of the whitish green calyxes which are joined in a bell-shaped. It had 5 stamens in a petal tube. The anther is yellow with a dark brown tip, cylinder shape and is 4-6 mm long. The fruit is a glossy green globose berry, 1.1-1.6 cm in diameter, and the ripe fruits are dull yellowish orange. The seeds are yellow or tan flat globose with 1-2 mm in diameter (38-42).

S. spirale is widely distributed in Asia (Burma, Bangladesh, Bhutan, India, Lao, Sulawesi, China, Thailand, Vietnam, and Indonesia) and Oceania (Australia) (38-39). In the north of Thailand, *S. spirale* is found in Chiang Rai, Maehongson, Chiang Mai,

Payao, Tak, Prae, Nan, Lumphun and Lumpang (43). The traditional uses of *S. spirale* have been reported. For food, the cooked young leaves and the raw or cooked berries are eaten, especially in Yunnan and India. The Adi tribes used the warm decoction of the fruits to relieve stomachache (44), while Akha people in Thailand and China drank hot tea from the leaves to regulate their body temperature (45). Moreover, the leaves have been reported to have killing activity against intestinal worms and used for the treatment beriberi and swollen stomach in Vietnam (46). In Assam, the roots have been used as a narcotic, diuretic and to relieve toothache (38,41,47). It also found that *S. spirale* root has been used as an anesthetic. In Laos, the broken bark is macerated in cold water and used as a febrifuge for adults and infants (38,41). Furthermore, the mucous from soaking the seeds has been used to poultice the wound and abscess (40,42). Yao tribes in Thailand chewed or decocted the barks and leaves of *S. spirale* for the treatment of cough, chest pain and out of breath from lung disease (42).

In 1985, Chakravarti *et al.* found glycosidic alkaloids in the bark and woody part of the roots of *S. spirale* (48). After that, other alkaloids have been isolated. Their structures and parts of use are shown in Table 2.1.

Table 2.1 Alkaloids and steroidal glycosides isolated from *S. spirale*

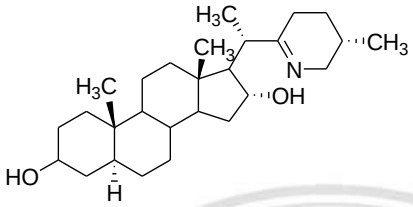
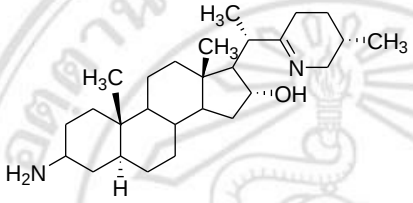
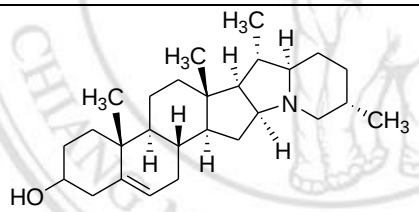
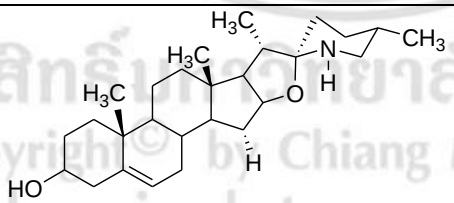
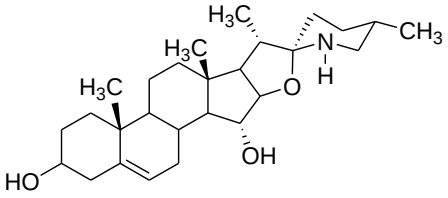
No.	Compounds	Plant part	Ref.
1*	 25-isosalafloridine	-	49
2*	 solacollinidine	-	49
3	 solanidine	leaves, fruits	50
4	 tomatidenol	leaves	51
5	 15α-hydroxytomatidenol	leaves	51

Table 2.1 (continued)

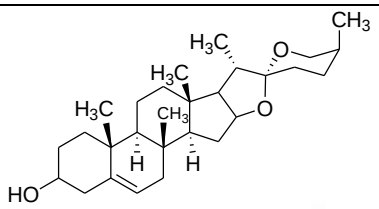
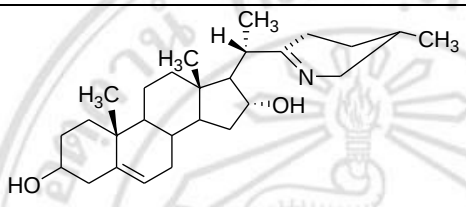
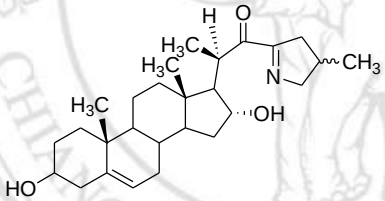
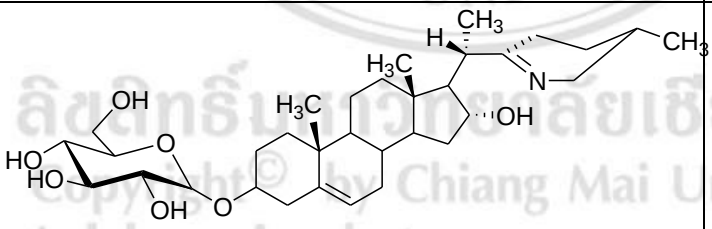
No.	Compounds	Plant part	Ref.
6	 yamogenin	leaves	51
7	 etioline	roots	51-52
8	 solaspiralidine	roots	52
9	 3-*O*-(*β*-D-glucopyranosyl) etioline	roots	52

Table 2.1 (continued)

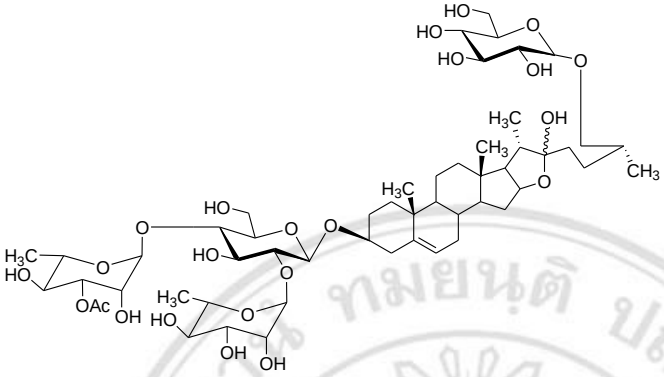
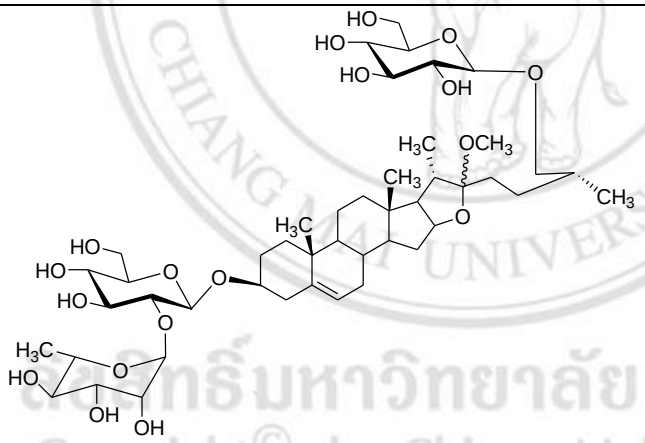
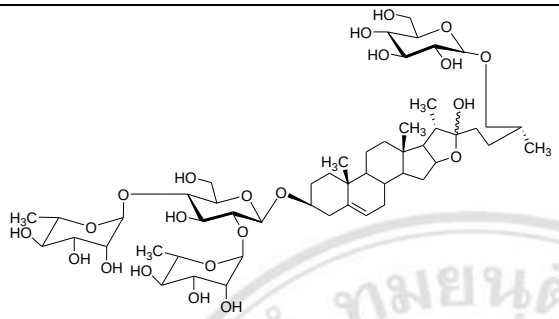
No.	Compounds	Plant part	Ref.
10	 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-3β,22ξ,26-triol-5-ene-3-O-α-L-rhamnopyranosyl-(1-2)-[(3-*O*-(3-*O*-acetyl)-α-L-rhamnopyranosyl-(1-4)]-β-D-glucopyranoside	fruits	46
11	 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-22ξ-methoxyl-3β,26-diol-5-ene-3-*O*-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside	fruits	46

Table 2.1 (continued)

No.	Compounds	Plant part	Ref.
12	 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-3β,22ξ,26-triol-5-ene-3-*O*-α-L-rhamnopyranosyl-(1-2)-[α-L-rhamnosyl-(1-4)]-β-D-glucopyranoside (protodioscin)	fruits	46

* 25-isosalafloridine and solacollinidine were also obtained from hydrolysis of the crude glycoalkaloid extract of *S. spirale* (53).

Most recently, Keawsa-ard *et al*. reported another three compounds (lupeol, protocatechuic acid and *trans*-cinnamic acid) which were isolated from the leaves of *S. spirale* (54) (Figure 2.4).

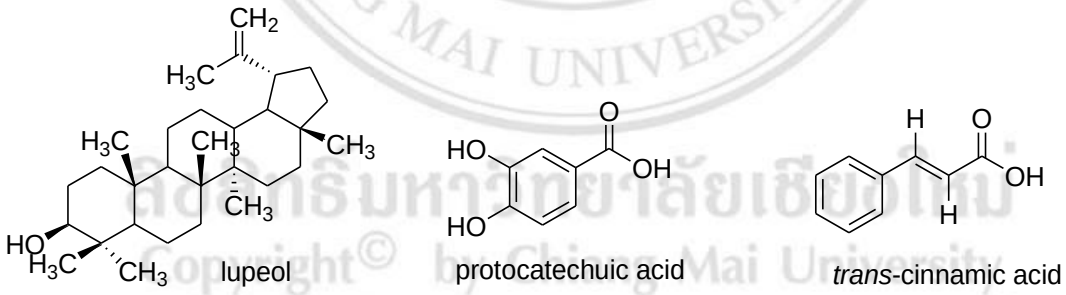


Figure 2.4 The structure of lupeol, protocatechuic acid and *trans*-cinnamic acid

Furthermore, the essential oil of the leaves of *S. spirale* was isolated by the hydrodistillation method and its composition was identified using GC-MS. The major compounds are (*E*)-phytol, β-selinene and α-selinene (55).

Some biological activities of *S. spirale* have been reported. Hu *et al.* found that the ethanolic and aqueous extracts of the stems of *S. spirale* showed no antifungal activity against *Pyricularia oryzae* (56). Keawsa-ard *et al.* revealed that the methanolic

extract of unripe fruits, the ethanolic extract of the flowers and the essential oil of *S. spirale* possessed antioxidant activities when determined by the DPPH assay (55,57). The antibacterial activities of *S. spirale* are shown in Table 2.2.

Table 2.2 The antibacterial activities of *S. spirale*

Extract	Plant part	MIC (mg/mL)		Ref.
		E. coli	*S. aureus*	
Hexane	Leaves	1500	1500	54
$CHCl_3$	Leaves	1500	375	54
Essential oil	Leaves	11.5	18.2	55
MeOH	Unripe friuts	375.0	187.50	57
EtOH	Flowers	48.88	488.88	57
EtOH	-	A*	NA*	58

*A = showed an inhibition zone without MIC value; NA = no activity.

Moreover, the anticancer activities of *S. spirale* have been reported and are summarized in Table 2.3.

Table 2.3 The anticancer activities of *S. spirale*

Extracts	Plant part	IC_{50} (μg/mL)[a]				Ref.
		KB[b]	MCF-7[b]	NCI-H187[b]	Vero	
Hexane	Leaves	-	-	-	-	54
$CHCl_3$	Leaves	42.73	17.90	36.74	-	54
Essential oil	Leaves	26.42	19.69	24.02	-	55
Hexane	Unripe fruits	-	-	-	NT	57
$CHCl_3$	Unripe fruits	-	-	29.23	NT	57
MeOH	Unripe fruits	-	-	-	NT	57
EtOH	Flowers	45.97	27.91	37.64	-	57

[a] (-) = no activity, NT = not tested; [b]KB = KB-Oral cancer, MCF-7 = MCF-7 Breast cancer, NCI-H187 = NCI-H187-Small cell lung cancer.

In 2014, Kalita *et al.* evaluated the nutrients of wild edible food plants in Northeast India, they found that the fruits of *S. spirale* contained high vitamin C and phosphorus, whereas the leaves of *S. spirale* had high vitamin E and potassium (59).

2.5 Taxa and Classification of *Gynura divaricata* (L.) DC. (60-61)

Kingdom: Plantae (Plants)

Subkingdom: Tracheophyta (Vascular plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Asterales

Family: Asteraceae (Aster family)

Genus: Gynura

Species: *Gynura divaricata* (L.) DC.

Synonyms (61)**:** *Cacalia albicans* Sessé & Moc., *Cacalia ovalis* Ker Gawl., *Gynura auriculata* Cass., *Gynura divaricata* subsp. *divaricata*, *Gynura glabrata* Hook.f., *Gynura hemsleyana* H. Lév., *Gynura incana* (L.) Druce, *Gynura ovalis* var. *ovalis*, *Gynura ovalis* var. *pinnatifida Hemsl*., *Senecio divaricatus* L.

Common names (62-64)**:** Khon Gai Thong Kum (Thai), Jīn jī máo yiè, Bai Bei San Qi, Bai Zi Cai (Chinese), Daun dewa (Indonesian), Daun dewa (Malay).

Figure 2.5 *Gynura divaricata* (L.) DC.

2.6 Review of traditional use, chemical composition and bioactivities of *G. divaricata*

Gynura divaricata (L.) DC., a Chinese traditional medicinal herb, is widely distributed in China, Hong Kong, Thailand, Indonesia, Malaysia and Vietnam. It is a perennial herb with erect stem which is 30-50 cm tall (Figure 2.5). The stem is light green to purple with upper synflorescence branch. The branches are easy to fracture. The leaves are simple, ovate, slightly serrate, acute, green, deep veins and shortly pubescent. These leave features are used to distinguish *G. divaricata* from *G. procumbens*. The leaves of *G. procumbens* are wide oval, slightly serrate, slightly serrate and soft pubescent like velvet. The flowers of *G. divaricata* are yellowish orange, small, scented and slightly exceeding involucres (62-64).

G. divaricata has anti-inflammatory, anti-swelling and blood purifying properties. The traditional uses of *G. divaricata* have been reported. It has been used for the treatment of bronchitis, pulmonary tuberculosis, pertussis, toothache, rheumatic arthralgia, uterine bleeding and diabetes. For external use, the macerated fresh herb or pulverized roots with sugar are topically applied in traumatic injury, fracture, wound bleeding, mastitis, leg ulcer, burns and scalds (63,65).

The chemical constituents of *Gynura divarica* (L.) DC. have been identified since 1996. The structure of each compound is shown in Table 2.4.

Table 2.4 The chemical constituents of *G. divaricata*

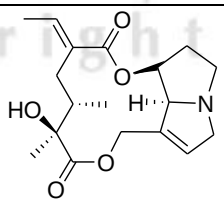
No.	Compounds	Plant part	Ref.
1	 Integerrimine	Aerial	66

Table 2.4 (continued)

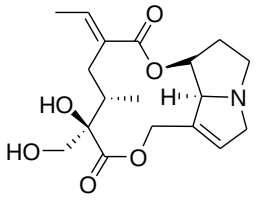
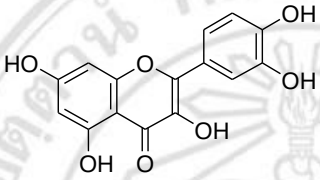
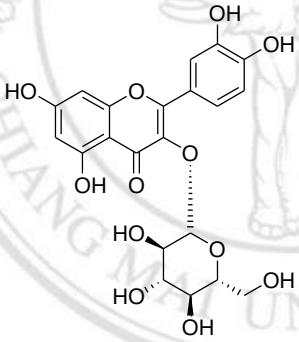
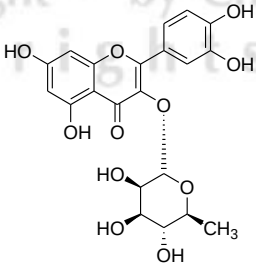
No.	Compounds	Plant part	Ref.
2	 usaramine	Aerial	66
3	 quercetin	Aerial	67,73,74
4	 quercetin 3-*O*-β-D-glucoside (Isoquercetin)	Aerial	67,73,74
5	 quercetin 3-*O*-α-L-rhamnopyranoside	Aerial	67,75

Table 2.4 (continued)

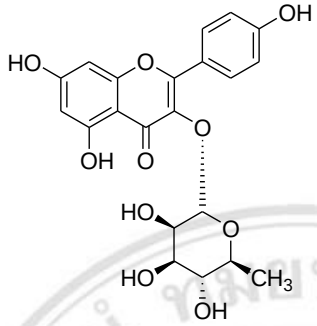
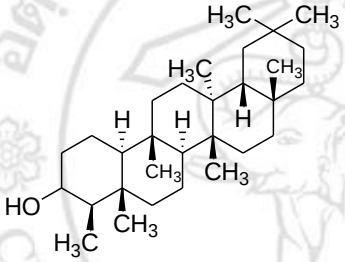
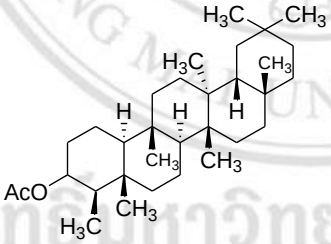
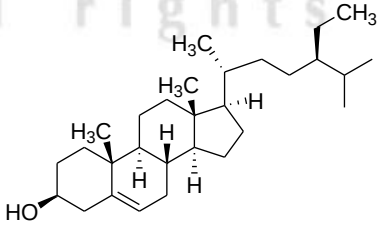
No.	Compounds	Plant part	Ref.
6	 kaemferol 3-*O*-α-L-rhamnopyranoside	Aerial	67,75
7	 *epi*-friedelanol	Aerial	67
8	 acetated *epi*-friedelinol	Aerial	67
9	 β-sitosterol	Aerial	67,68,71

Table 2.4 (continued)

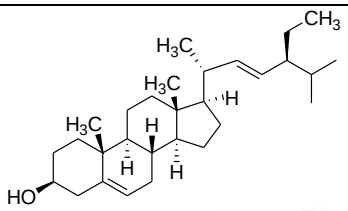
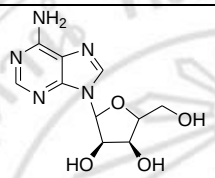
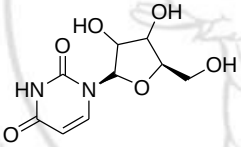
No.	Compounds	Plant part	Ref.
10	 stigmasterol	Aerial	67,68,71
11	 adenosine	Aerial	67,72
12	 uridine	Aerial	67,72
13	$H_3C-(CH_2)_{18}-CH_3$ eicosane	-	68,71
14	$HO-(CH_2)_{23}-CH_3$ tetracosanol	-	68
15	$HOOC-(CH_2)_{26}-CH_3$ octacosanoic acid	-	68
16	$HO-(CH_2)_{27}-CH_3$ octacosanol	-	68
17	$HOOC-(CH_2)_{14}-CH_3$ palmitic acid (hexadecanoic acid)	-	68

Table 2.4 (continued)

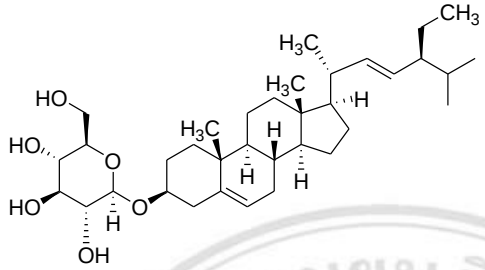
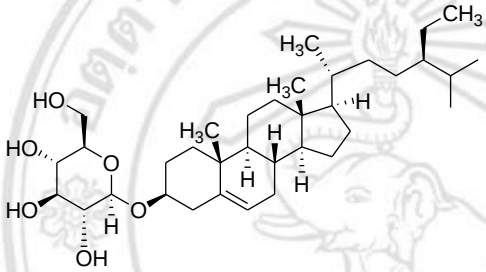
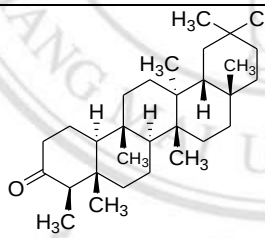
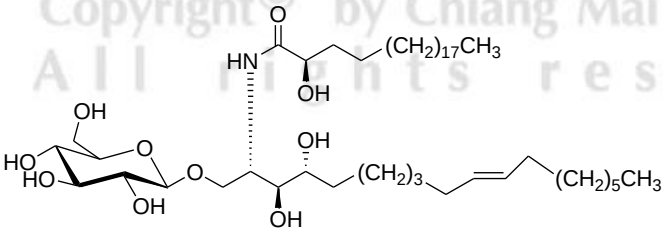
No.	Compounds	Plant part	Ref.
18	 stigmasterol-3-*O*-β-D-glucopyranoside	-	68,72
19	 daucosterol (sitosterol 3-*O*-β-D-glucopyranoside)	-	68,71
20	 friedeline	-	68
21	 1-*O*-β-D-glucopyranosyl-(2*S*, 3*S*, 4*R*, 10*E*)-2-[(2'*R*)-2'-hydroxyldocosanoyl-amino]-10-octadecene-1,3,4-triol (gynuraoside)	Aerial	63

Table 2.4 (continued)

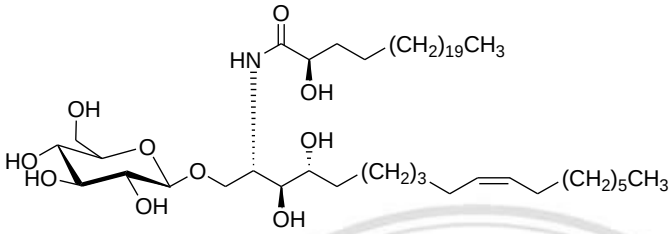
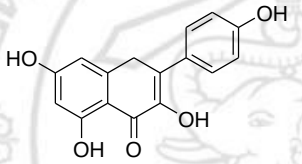
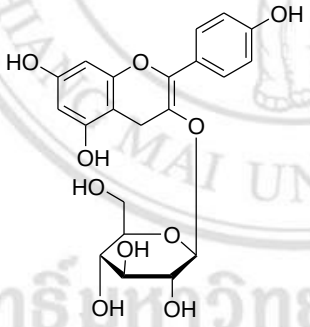
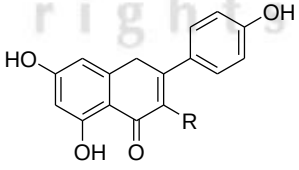
No.	Compounds	Plant part	Ref.
22	 1-*O*-β-D-glucopyranosyl-(2*S*, 3*S*, 4*R*, 10*Z*)-2-[(2'*R*)-2'-hydroxylignocenoyl-amino]-10-octadecene-1,3,4-triol	Aerial	69
23	 kaempferol	Aerial	69,71,73,74
24	 kaempferol-3-*O*-glucoside (Astragalin)	Aerial	69,71,73,74
25	 R = Glucose (6→1) rhamnose kaempferol 3-*O*-rhamnosyl(1→6)glucoside	Aerial	69

Table 2.4 (continued)

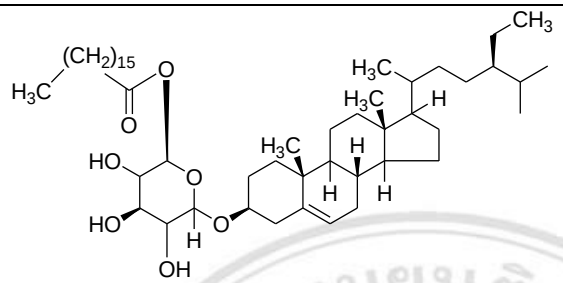
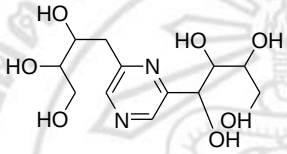
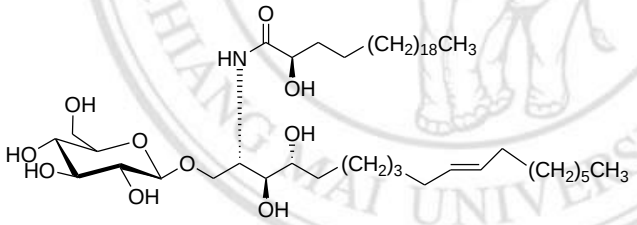
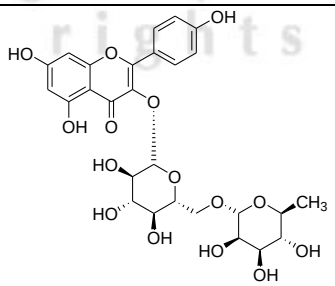
No.	Compounds	Plant part	Ref.
26	 β-sitosterol glucoside- 6'-O-heptadecoicate	Aerial	69
27	 2-(1', 2', 3', 4'-tetrahydroxybutyl)-6-(2", 3", 4"-trihydroxybutyl)-pyrazine	Aerial	69,72
28	 1-O-β-D-glucopyranosyl-(2S, 3S, 4R, 10S)-2-[(2'R)-2'-hydroxyltricosanoyl-amino]-10-octadecene-1,3,4-triol	Aerial	70
29	 kaempferol 3-O-rutinoside (nicotifiorin)	Aerial	71,73

Table 2.4 (continued)

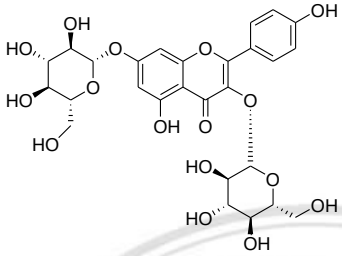
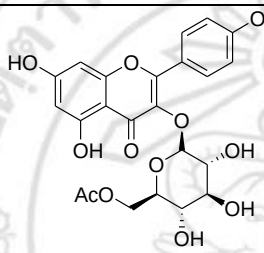
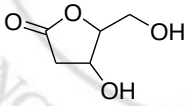
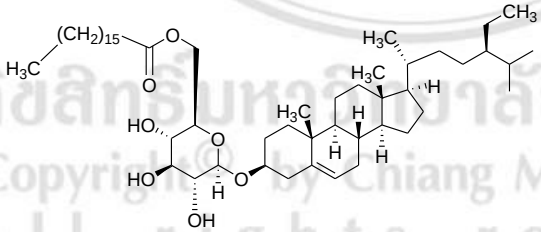
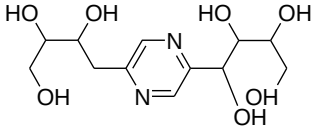
No.	Compounds	Plant part	Ref.
30	 kaempferol-3,7-di-O-β-D-glucopyranoside	Aerial	71,73
31	 kaempferol 3-O-(6''-O-acetyl)-β-D-glucopyranside	Aerial	71
32	 4-hydroxy-5-hydroxymethyl-γ-butyllactone	Aerial	71
33	 β-sitosterol glucoside-6'-O-heptadecanoate	Aerial	71
34	 2-(1', 2', 3', 4'-tetrahydroxybutyl)-5-(2'', 3'', 4''-trihydroxybutyl)-pyrazine	Aerial	72

Table 2.4 (continued)

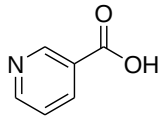
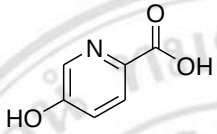
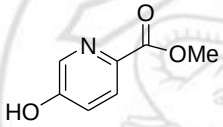
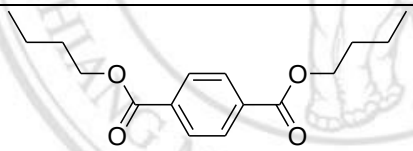
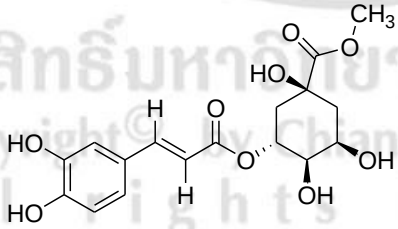
No.	Compounds	Plant part	Ref.
35	 nicotinic acid	Aerial	72
36	 5-hydroxy-piconilic acid	Aerial	72
37	 methyl-5-hydroxy-2-pyridinecarboxylate	Aerial	72
38	 dibutyl terephthalate	Aerial	72
39	 methyl chlorogenate	Aerial	72

Table 2.4 (continued)

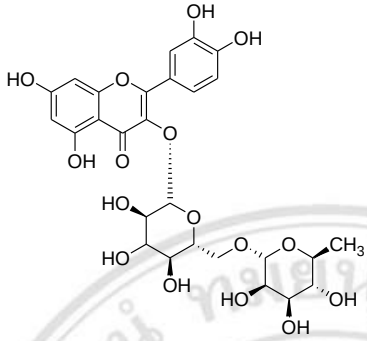
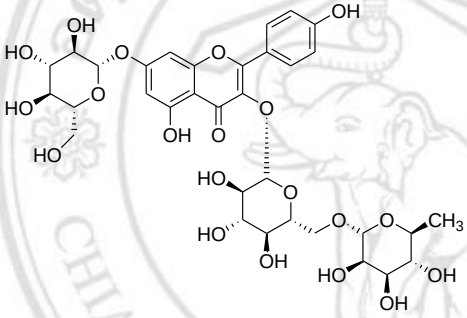
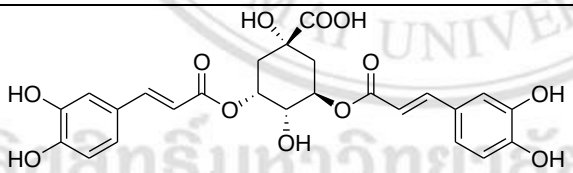
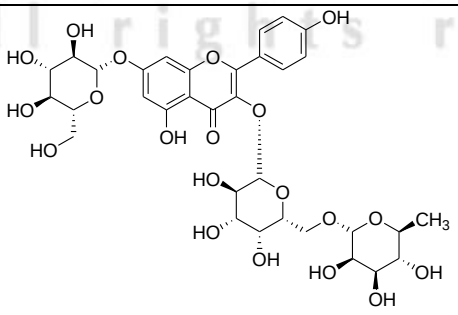
No.	Compounds	Plant part	Ref.
40	 quercetin-3-*O*-rutinoside (rutin)	Leaves	73,74
41	 kaempferol-3-*O*-rutinoside-7-*O*-β-D-glucopyranoside	Leaves	73
42	 3,5-dicaffeoylquinic acid	Leaves	73,76
43	 kaempferol 3-*O*-robinobioside-7-*O*-β-D-glucoside	Leaves	73

Table 2.4 (continued)

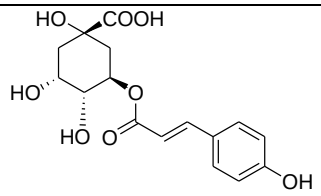
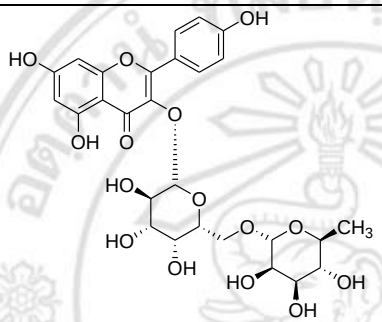
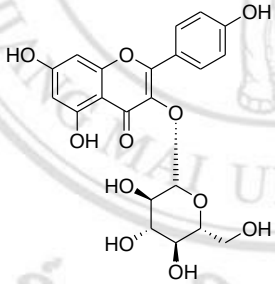
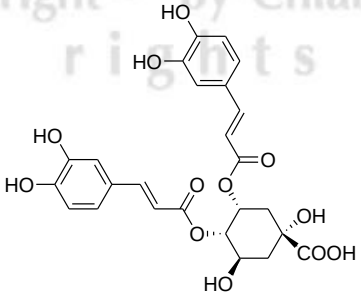
No.	Compounds	Plant part	Ref.
44	 p-coumaroylquinic acid	Leaves	73
45	 kaempferol 3-O-robinobioside	Leaves	73
46	 kaempferol 3-β-D-galactoside	Leaves	73
47	 3,4-dicaffeoylquinic acid	Leaves	73,76

Table 2.4 (continued)

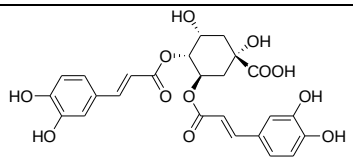
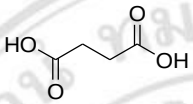
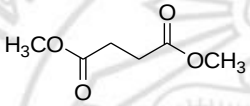
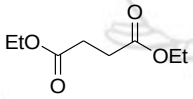
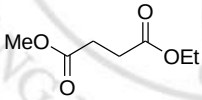
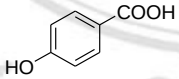
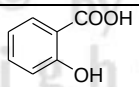
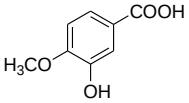
No.	Compounds	Plant part	Ref.
48	 4,5-dicaffeoylquinic acid	Leaves	73,76
49	 succinic acid	Aerial	74
50	 methyl succinate	Aerial	74
51	 ethyl succinate	Aerial	74
52	 ethyl methyl succinate	Aerial	74
53	 4-hydroxybenzoic acid	Aerial	74
54	 salicylic acid	Aerial	74
55	 Isovanillic acid	Aerial	74

Table 2.4 (continued)

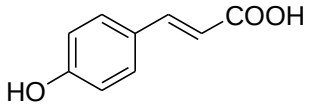
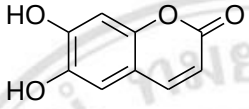
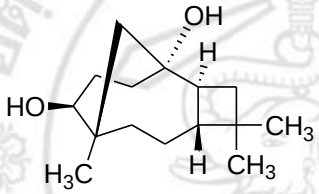
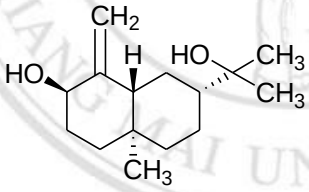
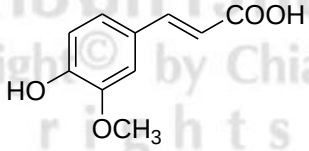
No.	Compounds	Plant part	Ref.
56	 p-coumaric acid	Aerial	74
57	 esculetin	Aerial	74
58	 caryolane-1,9β-diol	-	75
59	 eudesm-4(14)-ene-3a, 11-diol	-	75
60	 ferulic acid	-	75

Table 2.4 (continued)

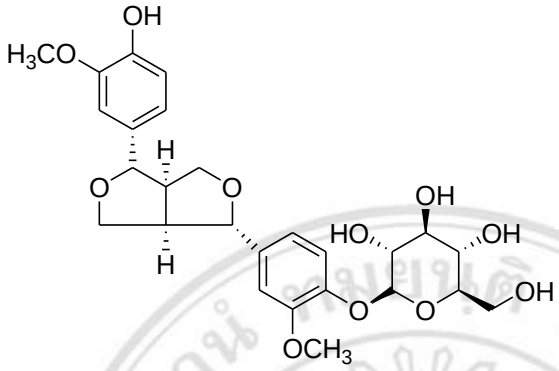
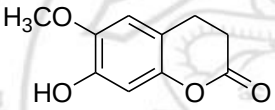
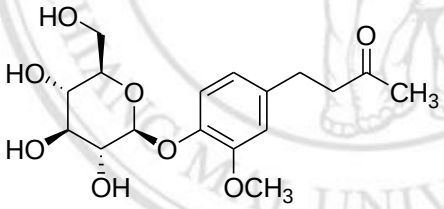
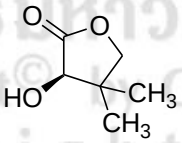
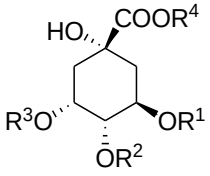
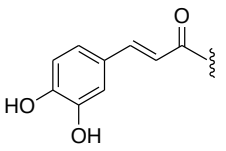
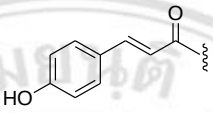
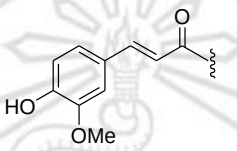
No.	Compounds	Plant part	Ref.
61	 pinoresino-4-*O*-β-D-glucopyranoside	-	75
62	 scopoletin	-	75
63	 zingerone-4-*O*-β-D-glucopyranoside	-	75
64	 (3*R*)-3-hydroxy-4,4-dimethyl-4-butyrolactone	-	75

Table 2.4 (continued)

No.	Compounds	Plant part	Ref.
65	 caffeoyl =  *p*-coumaroyl =  feruloyl =  1: R^1 = caffeoyl, R^2 = R^3= R^4 = H (5-*O*-caffeoylquinic acid) 2: R^1 = *p*-coumaroyl, R^2 = R^3= R^4 = H (5-*O*-*p*-coumaroylquinic acid) 3: R^1 = feruloyl, R^2 = R^3= R^4 = H (5-*O*-feruloylquinic acid) 4: R^1 = caffeoyl, R^2 = R^3= H, R^4 = Me (methyl 5-*O*-caffeoylquinate) 5: R^1 = H, R^2 = R^3= caffeoyl, R^4 = Me (methyl 3,4-dicaffeoylquinate) 6: R^1 = R^3 = caffeoyl, R^2 = H, R^4 =Me (methyl 3,5-dicaffeoylquinate) 7: R^1 = R^2 = caffeoyl, R^3= H, R^4 = Me (methyl 4,5-dicaffeoylquinate) 8: R^1 = R^2 = caffeoyl, R^3= H, R^4 = Et (ethyl 4,5-dicaffeoylquinate)	Aerial	76

The compositions of the essential oil from the leaves of *Gynura divaricata* (L.) DC. growing in China have been also studied. This study showed that the major constituents were τ–cadinene (20.8%) and γ–elemene (10.6%) (77). In 2012, Chen *et al*. reported that the major constituents of the essential oil, from *G. divaricata* growing in two different areas (Nanjing and Nanping) in the east of China, were, α–pinene (49.7%) and β–caryophyllene (43.7%), and β–caryophyllene (48.2%) and limonene (21.0%), respectively (78).

The biological activities of the plant extracts have been reported, the ethanolic and the aqueous extracts of *G. divaricata* showed good antioxidant activity (79-80). Moreover, the 50% ethanolic extract of *G. divaricata* showed 60% inhibition of *Streptococcus sobrinus* growth and 28% inhibition of glucosyltransferase (GTAase). (81) The extract of *G. divaricata* showed a protective effect in carbon tetrachloride-induced liver injury rats (82).

In addition, the aqueous extracts from the aerial part of *G. divaricata* decreased the levels of blood glucose in type-2 diabetic mice (83) and the aqueous and ethanol extracts of fresh plants had a significant effected on lowering the blood glucose level in normal mice, while the ethyl acetate and n-butanol extracts of the dried plants reduced blood glucose levels in both normal and alloxan-diabetic mice (84). The *in vitro* studies of *G. divaricata* extracts revealed a hypoglycemic effect via α-amylase and α-glycosidase which are the key enzymes relevant for type-2 diabetes. These extracts also significantly inhibited angiotensin-1 converting enzyme (ACE) that is involved in hypertension pathogenesis (85). The polysaccharides and flavonoids from *G. divaricata* possessed hypoglycemic effects in streptozotocin-induced diabetic rats and alloxan-induced diabetic mice (86-88).

Moreover, gynuraoside (compound No. 21, Table 2.4) exhibited strong cytotoxicity against L1210 murine leukemic cell growth (63). The caffeoylquinic acid derivatives from the aerial parts of *G. divaricata* have been tested for their inhibitory activities against yeast α-glucosidase and Protein Tyrosine Phosphatase 1B (PTP1B). The results showed that 3,4-dicaffeoylquinic acid (compound No. 47, Table 2.4), 4,5-dicaffeoylquinic acid (compound No. 48, Table 2.4), methyl 3,4-dicaffeoylquinate

(compound No. 65 (5), Table 2.4) and methyl 4,5-dicaffeoylquinate (compound No. 65 (7), Table 2.4) showed α-glucosidase inhibitory activities. Methyl 3,4-dicaffeoylquinate showed the highest activity with an IC_{50} value of 12.23 μM. While, 3,5-dicaffeoylquinic acid (compound No. 42, Table 2.4), 4,5-dicaffeoylquinic acid, methyl 3,4-dicaffeoylquinate and 5-*O*-*p*-coumaroylquinic acid (compound No. 65 (2), Table 2.4) had inhibitory effects on PTP1B. 4,5-Dicaffeoylquinic acid showed the highest activity with 58.2% PTP1B inhibition (76).

CHAPTER 2

Review of the literature

2.1 Taxa and Classification of *Stevia rebaudiana* (Bertoni) Bertoni (196-197)

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (Vascular plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteridae

Order: Asterales

Family: Asteraceae (Aster family)

Genus: *Stevia* Cav. (candyleaf)

Species: *Stevia rebaudiana* (Bertoni) Bertoni (candyleaf)

Common names (197): stevia, sweet leaf of Paraguay, caa-he-éé, kaa jheéé, ca-a-jhei, ca-a-yupi, azucacaa, eira-caa, capim doce, erva doce, sweet-herb, honey yerba, honeyleaf, yaa waan, candy leaf

Figure 2.1 *Stevia rebaudiana* Bertoni (198)

Stevia is a native herb to the northern area of South America and is still found growing in Brazil and Paraguay (199-200). The natural habitat of Stevia is a humid subtropical climate with the temperature range from 21-43 ºC. It grows faster at 21-24 ºC. It prefers sandy soils near a stream, acid infertile sands or mud soils and well-drained areas (198,201-202). Stevia is a perennial herb which grows up to 1 m. The leaves are simple, sessile, 3-4 cm long, spatulate or elongate-lanceolate shaped, serrate margin from the middle to the tip and entire beneath. The woody stem is weak-pubescent at the bottom. Its rhizome has lightly branching roots. White and small flowers with a light purple throat are pentamerous and are composite encircled by an involucre of epicalyx. The fruit is an achene with a five-ribbed spindle shaped (198,202).

2.2 Review of chemical constituents and bioactivities of *Stevia rebaudiana* Bertoni

The phytochemical constituents of Stevia were reported. The main phytochemical constituents of Stevia leaves were stevioside (4-13% w/w), followed by rebaudioside A (2-4% w/w), rebaudioside C (1-2% w/w) and dulcoside A (0.4-0.7% w/w), respectively. The minor constituents of the leave extracts were found as steviolbioside, rebaudioside B, D, E and F (Figure 2.2) (199-200).

Other labdane diterpenes have been isolated and characterized as jhanol, austroinulin, 6-*O*-acetylaustroinulin, *iso*-austroinulin, sterebin E, sterebin E acetate, and sterebin A acetate (199,203). The triterpenes isolated include, β-amyrin acetate and lupeol, while the sterols (β-sitosterol, stigmasterol and campesterol) were also obtained from Stevia leaves (204).

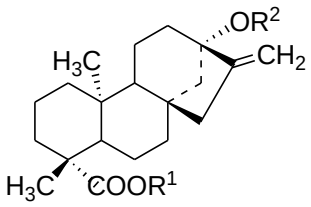
A preliminary phytochemical screening of the leaf powder showed the most abundant compounds to be tannins, followed by alkaloids, sterols and triterpenes saponins, cardiac glycosides, and anthraquinones (205).

Tadhani and Subhash (206) analyzed the water extract (WE), and methanol-water (50/50 v/v) extract (MWE) of Stevia leaves using RP-HPLC. They showed that the major compounds were protocatechuic acid (1.46 mg/mL), catechin (1.44 mg/mL) and

quercetin glucosyl (1.19 mg/mL), whereas the MWE exhibited the major compounds as quercetin dihydrate (4.48 mg/mL), rutin (1.99 mg/mL) and chlorogenic acid (1.03 mg/mL).

The essential oil of Stevia was also analyzed. The major constituents were β-caryophyllene, *trans*-β-farnesene, α-humulene, δ-cadinene, caryophyllene oxide and nerolidol, an unidentified alcohol, and linalool, terpinen-4-ol and terpineol (207). In a recent study, the essential oil of Stevia powder was identified and analyzed by GC-MS. The major components were carvacrol, caryophyllene oxide, spathulenol and cardinol (206).

Michalik *et al.* (189) isolated and purified (−)-steviamine from Stevia leaf extract. It is the first polyhydroxylated indolizidine alkaloid to have a methyl group at C-5 and a hydroxymethyl group at C-3.



Compounds	R^1	R^2
Steviol	H	H
Steviolbioside	H	β-Glc-β-Glc(2→1)
Stevioside	β-Glc	β-Glc-β-Glc(2→1)
Rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside C	β-Glc	β-Glc-α- Rha(2→1) | β-Glc(3→1)
Rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
Rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
Rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
Dulcoside A	β-Glc	β-Glc-α- Rha(2→1)

Figure 2.2 Structures of some chemical constituents of *Stevia rebaudiana* Bertoni leaves; Glc, Xyl, and Rha represent glucose, xylose and rhamnose sugar moieties, respectively (202)

The biological activities of Stevia extracts and pure compounds were investigated.

2.2.1 Antimicrobial activity

The antibacterial screening of the essential oil (EO), water extract (WE) and methanol-water extract (WME) of Stevia leaves using the disk-diffusion method were examined against the bacteria, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* and the fungi, *Aspergillus niger* and *Candida albicans*. The results showed that all samples exhibited inhibitory activities against all the tested microorganisms except the EO and the WE which were inactive against *P. aeruginosa* and *A. niger*, respectively (206).

The *in vitro* antimicrobial activities of the hexane, ethyl acetate, methanol and water extracts of dried Stevia leaves were investigated using the cup plate method. The selected microorganisms were *B. subtilis, S. aureus, Micrococcus luteus, Serratia marcescens, P. aeruginosa, Bacillus megaterium, E. coli, Proteus vulgaris* and yeast: *A. niger and Rhizopus oligoporus*. The results showed that only the water extract possessed antimicrobial activity against *B. subtilis* and *S. aureus* whereas the other extracts inhibited all microorganisms (208).

The antibacterial activities of the water, acetone, ethyl acetate and chloroform extracts of Stevia leaves against *S. aureus, Salmonella typhi, E. coli, B. subtilis, Aeromonas hydrophila* and *Vibrio cholerae* using the agar well diffusion method and the anti-yeast and antifungal activities of these extracts against *C. albicans, Cryptococcus neoformans, Trichophyton mentagrophytes* and *Epidermophyton species* were also tested. The results revealed that the acetone extract showed the greater antibacterial activity, while the water extract was inactive against all bacterial strains. All the extracts showed an inhibitory effect on the growth of *Epidermophyton species* and *C. albicans* (209).

2.2.2 Cytotoxic activity

The cytotoxicity of the aqueous, ethyl acetate, acetone and the dilution of acetone extracts of Stevia leaves against Vero and HEp2 (Human laryngeal epithiloma cells) cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT] assay. The results showed that the aqueous extract was inactive to tumor cells except the acetone and ethyl acetate extracts which showed more cytotoxicity against HEp2 cells. The acetone extracts exhibited the highest cytotoxicity followed by the ethyl acetate and chloroform extracts. Among the 1:2, 1:4 and 1:8 dilutions of the acetone extracts, the 1:8 dilution was non-toxic to Vero cells and also showed the cytotoxicity and anti-proliferative activities against HEp2 cells (209).

Furthermore, stevioside showed cytotoxicity against MCF-7 (human breast cancer cell), including the inhibition of cell proliferation, suppression of cell viability and the induction of apoptosis via a mitochondrial apoptotic pathway (210).

2.2.3 Antiviral activity

The analysis of anti-rotavirus activity of the hot water extract from dried stems and leaves of Stevia was determined by the MTT assay. This extract inhibited plaque formation of four serotypes of human rotavirus (HRV) *in vitro* (211).

The crude aqueous extract, the alkaline extract and the arabinogalactan from Stevia leaves showed antiviral activity against Herpes Simplex virus type-1 (HSV-1) *in vitro* (212).

2.2.4 Antidiabetic activity

The *in vivo* antihyperglycemic effect of stevioside in type 2 diabetic Goto-Kakizaki (GK) rats was investigated. An i.v. glucose tolerance test (IVGT) was performed with and without stevioside. It was reported that stevioside exhibited antihyperglycemic, insulinotropic and glucanostatic actions which may possess

the potential to be a new antidiabetic agent for the treatment of type 2 diabetes (202).

A major constituent of Stevia, rebaudioside A (Reb A), was evaluated for antihyperglycemic activity in the streptozotocin (STZ)-induced diabetic rats. Oral treatment with Reb A (50, 100, and 200 mg/kg) daily for 45 days to these diabetic rats significantly reduced the blood glucose level ($P<0.05$) and significantly increased the plasma insulin level ($P<0.05$) when compared with the diabetic rats (213).

In an acute, paired cross-over study, twelve patients with type 2 diabetes mellitus consumed a meal supplemented with either 1 g of stevioside or 1 g of a maize starch control and their blood glucose levels were monitored at 30 min. before and for 240 min. after intake of the test meal. It was found that stevioside significantly decreased the post prandial blood glucose level in the treatment group, suggesting for further investigation of its advantages in treatment of type 2 diabetes (214).

2.2.5 Hypoglycemia action

Stevioside (0.5 mg/kg) has ability to control the levels of blood glucose by enhancing insulin secretion and insulin utilization in two diabetic rat models (STZ-induced diabetic rats and NIDDM (noninsulin-dependent diabetes mellitus) diabetic rats induced by feeding with fructose) (199).

Furthermore, eighty Wistar rats were randomized to 8 groups. A standard control diet was separately added with either Stevia whole leaf powder or polyphenols or the fiber extracted from Stevia. They were fed for one month. Then, streptozotocin was injected to the diabetic groups on the 31st day. Several parameters were analyzed to evaluate the regulation of the streptozotocin induced oxidative stress, blood glucose levels and toxicity by Stevia. It was found that Stevia leaf powder and Stevia leaf polyphenols decreased the blood glucose levels and increased the insulin level when compared to the control diabetic rats (215).

2.2.6 Hypertension

The effect of intravenous injection (i.v.) of stevioside in the anesthetized SHRs (spontaneously hypertensive rats) on the blood pressure was studied. It was effective in systolic and diastolic blood pressure reduction and there was no change in serum catecholamines in anesthetized animals, while the another study found that intraperitoneal injection (i.p.) of stevioside (25 mg/kg) also exhibited an antihypertensive effect in SHRs (198).

A multicenter, randomized, double-blind, placebo-controlled study in 106 hypertensive Chinese subjects (age from 28-75 years) was undertaken. Two groups were divided, 60 subjects were given 250 mg stevioside capsules and 46 subjects received placebo three times daily and monitored at monthly intervals for one year. After three months, the researcher found that the systolic and diastolic blood pressure of the stevioside group significantly reduced and these effects continued during the whole year (216).

2.2.7 Anti-inflammatory and immunomodulatory activities

The methanol and chloroform extracts of Stevia leaves effected marked inhibition of carrageenan-induced paw edema in rats which showed a significant anti-inflammatory effect (203).

Stevioside (1 mM) significantly reduced the production of TNF-α (tumor necrosis factor alpha) and IL-1β (interleukin-1 beta) and slightly reduced nitric oxide (NO) production in lipopolysaccharide (LPS) - stimulated THP-1 cells without any direct toxic effects, whereas steviol (100 μM) did not. The activation of IKK-β (inhibitor of nuclear factor kappa-B kinase subunit beta) and transcription factor NFκB (Nuclear Factor-Kappa B) were suppressed by stevioside, as indicated by western blotting. This research showed that stevioside modurates the synthesis of the inflammatory mediators in LPS-stimulated THP-1 cells by interfering with the IKKβ and NFκB signaling pathway and stevioside increased TNF-α secretion is partly mediated through TLR-4 (Toll-like receptor 4) (217).

Stevioside was also tested as an immunostimulator on various parameters of the immune system, which were humural antibody titer, DTH (delayed type hypersensitivity) response, macrophage phagocytic response and lymphocytic proliferation, at three different doses (6.25, 12.5 and 25 mg/kg p.o.) on normal and cyclophosphamide treated mice. The result showed that administrating stevioside orally at the concentration of 12.5 mg/kg increased antibody production and DTH response, enhanced phagocytic function and substantially modulated T and B cell proliferation. This study revealed that stevioside possesses immunomodulatory activities (218).

2.2.8 Antioxidant activity

The extract of Stevia leaves showed a high degree of antioxidative activity and has been also revealed to inhibit the formation of hydroperoxide in sardine oil with a higher efficacy than that of either green tea extract or DL-α-tocopherol (202).

The antioxidant activities of the essential oil (EO), methanol-water extract (WME) and water extract (WE) of Stevia leaves have been determined by the DPPH (1-1-diphenyl 2-picryl hydrazyl) assay. The IC_{50} values of the EO, WE and MWE were 2.9, 5.0 and 9.26 µg/mL, respectively which indicated that Stevia extracts possess high antioxidant properties (206).

An *in vitro* study of the ethanolic and aqueous extracts of Stevia leaves, which contained higher levels of total phenolic compounds, showed strong antioxidant activity by inhibiting DPPH radical, hydroxyl radical, nitric oxide radical and superoxide radical scavenging activities when compared with standard ascorbic acid. These studies indicated that Stevia extracts have a significant efficacy for using as a natural antioxidant (219-220).

2.2.9 Toxicology

Subacute toxicity studies on rats which were fed with stevioside up to 7.0% concentration over a 50-day period have found no remarkable toxic effects (200).

The acute toxicity of stevioside was studied. Single doses of stevioside were given to rodents; the results exhibited no lethality within 14 days after the experiment and the clinical signs of toxicity, histopathological or morphological variation were not found (198).

Wistar rats were given with 4 mg/mL of the stevioside solution via oral administration (*ad libitum*) and the DNA-induced damage was measured using the comet assay (single cell gel electrophoresis). The results exhibited that stevioside produced lesions at different levels in peripheral blood, spleen, liver and brain cells. Therefore, these data pointed to the possible mutagenic properties of stevioside (221).

2.3 Indolizidine alkaloids

The indolizidine alkaloid structure consists of a fused piperidine-pyrrolidine bicyclic structure with N at the bridgehead (Figure 2.3) (192).

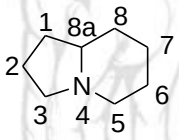


Figure 2.3 The core structure of indolizidine alkaloids

The naturally occurring indolizidine alkaloids have been isolated from various sources such as *Dendrobates* (poison frogs), *Mantella* (Madagascan frogs), *Tetramorium electrum* (ants), the Convolvulaceae and the Leguminosae family (222) (plants), *Rhizoctonia leguminicola* (fungus) (223), the genus of *Dendrobium* (orchids) (224), the genus of *Elueocarpus* (223) and *Tylophora* (224) (plants). Some examples of known indolizidine alkaloids are shown in Table 2.1.

Table 2.1 Examples of the known indolizidine alkaloids

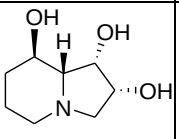
Compounds	structure	Parts of use	Source /Species (family)	Ref.
swainsonine [**2**]		leaves	*Swainsona canescens* (Leguminosae)	198

Table 2.1 (continued)

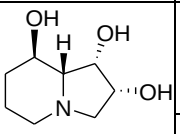
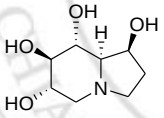
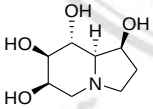
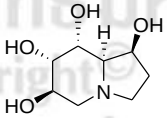
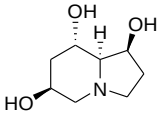
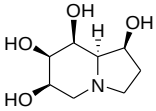
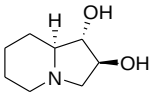
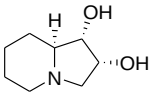
Compounds	structure	Parts of use	Source/Species (family)	Ref.
swainsonine [**2**]		leaves/stems	*Astragalus* spp. (Leguminosae)	192,225
		leaves/stems	*Oxytropis* spp. (Leguminosae)	192,226
		-	*Rhizoctonia leguminicola* (Basidiomycetes)	192
		-	*Metarhizium anisopliae* (Deuteromycetes)	192,227
		seeds	*Ipomoea* sp. aff. *calobra* (Convolvulaceae)	192
		leaves/stems	*Ipomoea carnea* (Convolvulaceae)	192,228
castanospermine [**3**]		seeds/leaves/ bark	*Castanospermum australe* (Leguminosae)	192,229
		seeds/leaves/ bark	*Alexa* spp. (Leguminosae)	192
6-*epi*-castanospermine		seeds/leaves/ bark	*Castanospermum australe* (Leguminosae)	192,229
6,7-di*epi*-castanospermine		seeds	*Castanospermum australe* (Leguminosae)	192
7-deoxy-6-*epi*-castanospermine		seeds	*Castanospermum australe* (Leguminosae)	192
6,8-di*epi*-castanospermine		Leaves and twigs	*Castanospermum australe* (Leguminosae)	191

Table 2.1 (continued)

Compounds	structure	Parts of use	Source/Species (family)	Ref.
lentiginosine [**4**]		leaves	*Astragalus lentiginosus* (Leguminosae)	192
2-*epi*-lentiginosine		-	*Rhizoctonia leguminicola* (Basidiomycetes)	192
		leaves	*Astragalus lentiginosus* (Leguminosae)	192,228

2.4 Polyhydroxylated alkaloids

Polyhydroxylated alkaloids could be isolated from microorganisms and plants and many have been studied as glycosidase inhibitors. Glycosidases are enzymes which catalyze the glycosidic bonds hydrolysis in the carbohydrate complexes and glycoconjugates (192). The various functions of glycosidases are involved in the survival and living of all organisms. For example, the intestinal oligo- and disaccharidases (e.g. sucrase, maltase, isomaltase, lactase, trehalase and hetero-β-glucosidase) digest large sugar-containing molecules to monosaccharides which are more easily absorbed through the surface of the epithelial cell of the brush border in the small intestinal (191); the α-mannosidase catabolises the glycoconjugates in lysosome (192) and the α-glucosidases and α-mannosidases trim the *N*-linked glycoproteins biosynthesis in the endoplasmic reticulum (ER) (230). The glycosidase inhibitions can also alter cell-cell or cell-virus recognition processes and showed the effects on the transport and the secretion of glycoproteins, the maturation and carbohydrate catabolism. The potential effects of glycosidase inhibitors have been applied as therapeutics of many diseases such as antidiabetic, antiviral and genetic disorders. The structures of polyhydroxylated alkaloids are similar to the sugar (pyranosyl or furanosyl) moiety of the natural substrates of glycosidase enzymes. Most of the polyhydroxylated alkaloids act as potent and specific glycosidase inhibitors. Therefore, the polyhydroxylated alkaloids are interesting target molecules for new therapeutic applications (192). The glycosidase-inhibiting polyhydroxylated alkaloids are classified

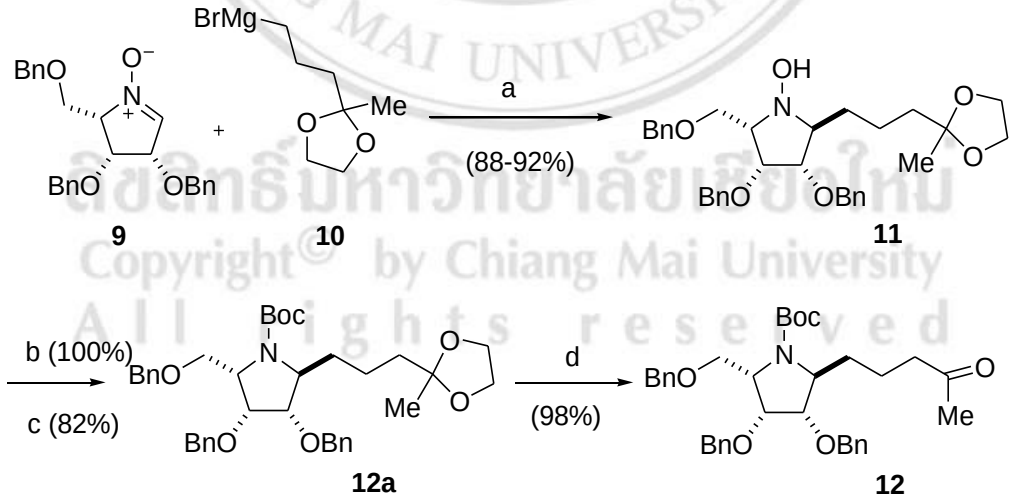
into five groups based on their structures; pyrrolidines, piperidines, indolizidines, pyrrolizidines and nortropanes (191).

The polyhydroxylated indolizidine alkaloid, swainsonine [**2**] was shown to be a strong and specific inhibitor of lysosomal acid and cytocysolic α-mannosidases and Golgi α-mannosidase II (192,231-233). Swainsonine has been reported to have anticancer activity. It can inhibit tumor growth and tumor cell proliferation, reduce metastasis and increase cellular immune responses (232-233). Castanospermine [**3**] was found to be a potent inhibitor of β-glucocerebrosidase, β-xylosidase, α-glucosidases (including amyloglucosidase, amylase, isomaltase, maltase, trehalase, sucrase, glucosidase I and II) and β-glucosidases (including cellobiase and lactase) (192,234). The α-glucosidase inhibitor property of castanospermine has been reported to be responsible to its antiviral activities against various viruses for examples; human immunodeficiency virus (HIV), human cytomegalovirus (CMV) (192,235), dengue virus (236) and human parainfluenza virus type 3 (HPIV3) (237). Lentiginosine [**4**] has been reported as an amyloglycosidase inhibitor (192,238). 6-*Epi*-castanospermine was also found to be an amyloglucosidase inhibitor and a neutral α-mannosidase inhibitor. 6,7-Di*epi*-castanospermine was shown to be an amyloglucosidase inhibitor and fungal β-glucosidase inhibitor, while 7-deoxy-6-*epi*-castanospermine inhibits amyloglucosidase and yeast α-glucosidase (192). Natural (–)-steviamine exhibited a weak inhibition of β-glucosidases (from almond) with an IC_{50} value of 454 μM and against β-glucosidases (from *Caldocellum saccharolyticum*) with an IC_{50} value of 739 μM but it had a good inhibitory activity against β-galactosidase (from rat intestinal lactase) with an IC_{50} value of 35 μM. (–)-Steviamine also shows weak inhibition of *N*-acetyl-α-D-galactosaminidase (GalNAcase) with an IC_{50} value of 814 μM. There has been no previous report of any natural product which inhibited any GalNAcase. The GalNAcase inhibition may allow the design of chaperones for the treatment of Schindler-Kanzaki disease and may useful for cancer treatment by the protection of macrophage activating factor (193).

2.5 Synthesis of steviamine and its analogues and their glycosidase inhibitory activities

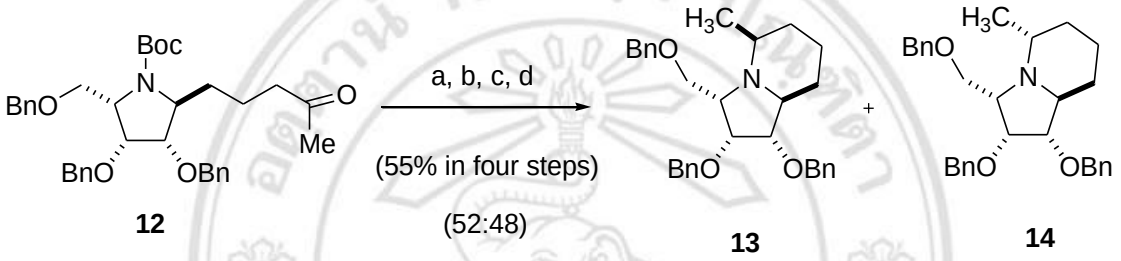
In 2009, Thompson *et al.* reported the X-ray crystallography structure of steviamine as its hydrobromide salt form, (1*R*,2*S*,3*R*,5*R*,8a*R*)-3-hydroxymethyl-5-methyloctahydroindolizine-1,2-diol hydrobromide, which indicated the absolute and relative stereochemistry at the five chiral centers (190). Steviamine [**1**] was announced as a new class of indolizidine alkaloid which was isolated from Stevia leaf extract and is also found in the leaves and the bulbs of *Veltheimia capensis*. Its NMR spectroscopic data and some physical properties were revealed (13). Since then steviamine [**1**] and its analogues have been of interest for organic synthesis.

In 2010, Hu *et al.* (193) reported the synthesis of the enantiomer of the natural (−)-steviamine and its C5 epimer. They first synthesized the key intermediate [**12**] starting from the D-ribose-derived cyclic nitrone [**9**] and Grignard reagent [**10**] via the diastereoselective addition to provide the hydroxylamine [**11**]. Reduction of [**11**] with Zn-Cu(OAc)$_2$-AcOH, followed by treating with (Boc)$_2$O generated the *N*-Boc derivative [**12a**]. Liberation of the carbonyl group of [**12a**], under mild acidic conditions, gave the key intermediate, the *N*-Boc ketone [**12**] (Scheme 2.1).



Scheme 2.1. *Reagents and conditions*: (a) THF, 0 ºC, 0.5 h; (b) Zn/Cu(OAc)$_2$/AcOH, rt, 24 h; (c) (BoC)$_2$O, 1N NaOH, rt, 12 h; (d) PPTS, acetone/H$_2$O, 65 ºC, 4 h.

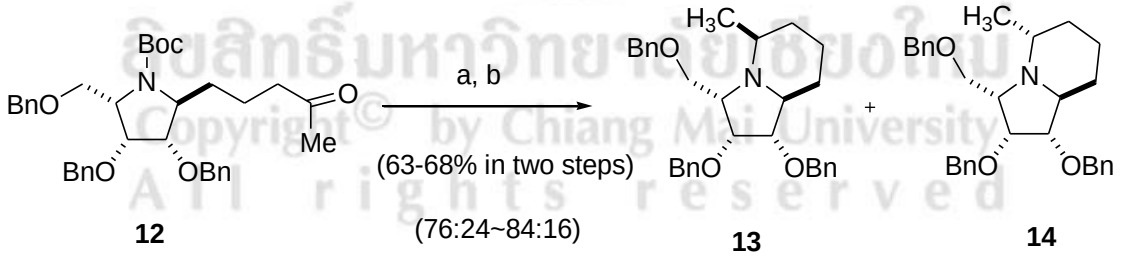
Two parallel annulation approaches were used for constructing the second ring. The first approach involved an intramolecular nucleophilic displacement reaction. This started with the reduction of the *N*-Boc ketone [**12**] with $NaBH_4$ to generate a mixture of diastereomeric alcohols, which were directly transformed to their corresponding mesylates without any purification. Then liberation of the amino group by CF_3CO_2H deprotection followed by treatment of the resulting amine with K_2CO_3 in methanol with a catalytic amount of water provided the two epimeric indolizidines [**13**] and [**14**] in nearly a 1:1 ratio (Scheme 2.2).



12 **13** **14**

Scheme 2.2. *Reagents and conditions*: (a) $NaBH_4$, MeOH, 0 ºC, 1 h; (b) MsCl, NEt_3, DCM, 0 ºC, 0.5 h; (c) CF_3CO_2H, DCM, 0 ºC, 1 h; (d) K_2CO_3, MeOH, rt, 24 h.

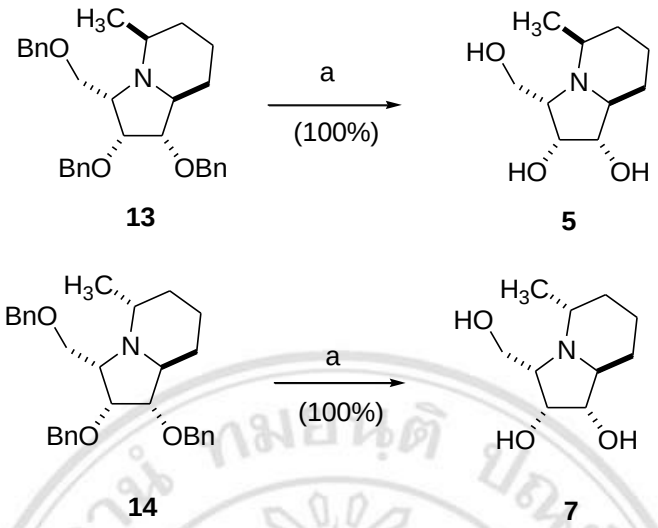
The second approach involved an intramolecular reductive amination reaction. This started with the acidic deprotection of compound **12** followed by the reduction of the iminium intermediate with $NaBH_4$ to form a mixture of the two epimers [**13**] and [**14**] in good yields (Scheme 2.3).



12 **13** **14**

Scheme 2.3. *Reagents and conditions*: (a) CF_3CO_2H, DCM, 0 ºC, 2 h; (b) $NaBH_4$, MeOH, 0 ºC, 1 h.

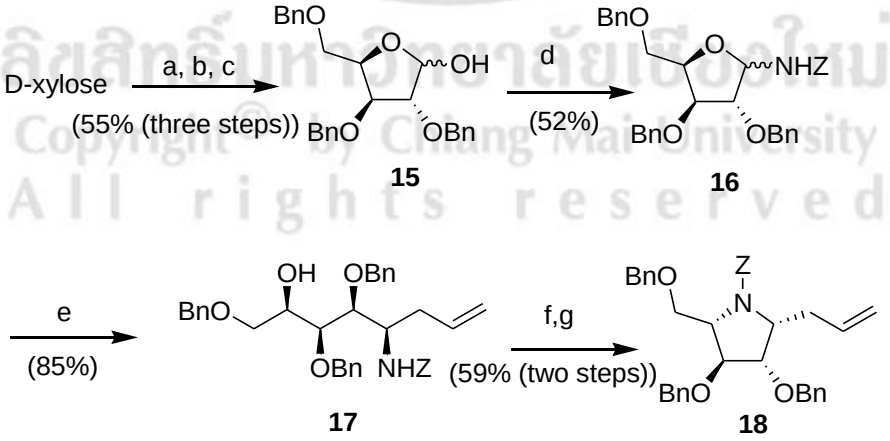
Finally, the compounds [**13**] and [**14**] were converted to (+)-steviamine [**5**] and 5-*epi*-(+)-steviamine [**7**] through hydrogenolysis reaction in quantitive yields, respectively (Scheme 2.4).



Scheme 2.4. *Reagents and conditions*: (a) Pd/C, H_2, MeOH, 6N HCl, rt, 16 h.

(+)-Steviamine [**5**] and 5-*epi*-(+)-steviamine [**7**] were found to have weak inhibitory activity against α-rhamnosidase (from *Penicillium decumbens*) with IC_{50} values of 484 and 342 μM, respectively.

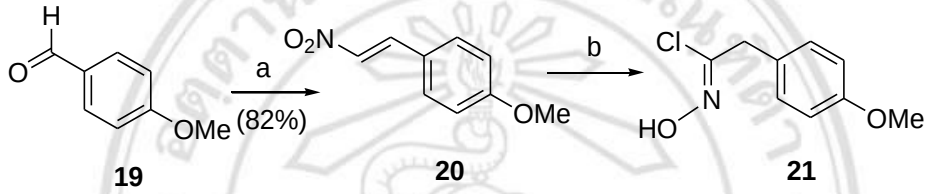
Chronowska *et al.* (195) accidentally synthesized an analogue of (–)-steviamine while making simple UDP-Galf analogues by the 1,3-dipolar cycloaddition reaction between a 1-C-allyl iminosugar and a nitrile oxide. Their synthesis began with the synthesis of the starting material, β-1-C-allyl-1,4-dideoxy-1,4-imino-L-arabinitol [**18**], in seven steps from D-xylose (Scheme 2.5).



Scheme 2.5. *Reagents and conditions*: (a) HCl (0.11 M), MeOH, 30 ºC, 3.5 h; (b) NaH

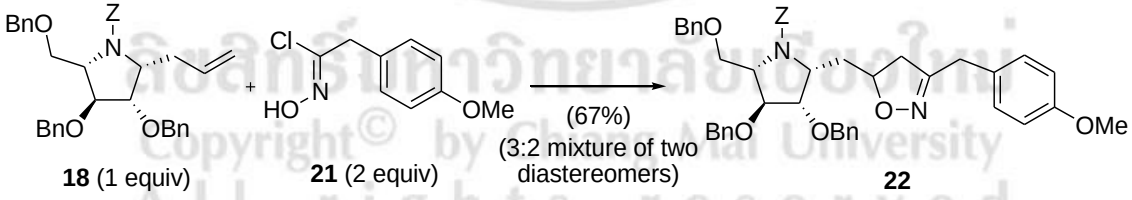
(6 equiv), BnBr (4.5 equiv), DMF, 0 ºC to rt; (c) 1 M HCl:AcOH (1:4), 80 ºC; (d) H_2NCO_2Bn (2 equiv), TMSOTf (1 equiv), CH_2Cl_2, 4 Å MS, rt; (e) AllTMS (7 equiv), TMSOTf (1 equiv), CH_3CN, –20 ºC; (f) MsCl (2.1 equiv), NEt_3 (2.2 equiv), CH_2Cl_2, 4 Å MS, rt; (g) *t*BuOK (2 equiv), THF, rt.

They then prepared the nitrile oxide moiety by converting of *p*-anisaldehyde [**19**] into the conjugated nitrostyrene compound [**20**], and then reacted this compound with titanium chloride in the presence of triethylsilane to obtain the expected hydroximoyl chloride [**21**] (Scheme 2.6).



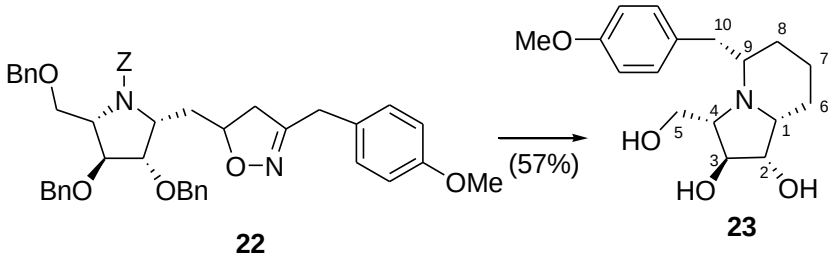
Scheme 2.6. *Reagents and conditions*: (a) $AcONH_4$ (1.1 equiv), CH_3NO_2, reflux; (b) Et_3SiH (2.1 equiv), $TiCl_4$ (2.2 equiv), CH_2Cl_2, rt, crude.

The 1,3-dipolar cycloaddition reaction was performed at room temperature by mixing the 1-C-allyl iminosugar [**18**] with an excess amount of the hydroximoyl chloride [**21**], generated in situ from nitrile oxide in the presence of triethylamine, to give compound [**22**] (Scheme 2.7).



Scheme 2.7. Reagents and conditions: NEt_3 (6 equiv), CH_2Cl_2, rt.

The final step was the reductive cleavage of the isoxazoline and the iminosugar deprotection of compound [**22**] through catalytic hydrogenolysis under aqueous acidic conditions to give directly the (–)-steviamine analogue [**23**] as a single diastereoisomer in a good yield (Scheme 2.8).



Scheme 2.8. *Reagents and conditions*: H_2, 10% Pd/C, H_2O (2 equiv), 1N HCl (1 equiv), MeOH/CH_2Cl_2 1:1, rt.

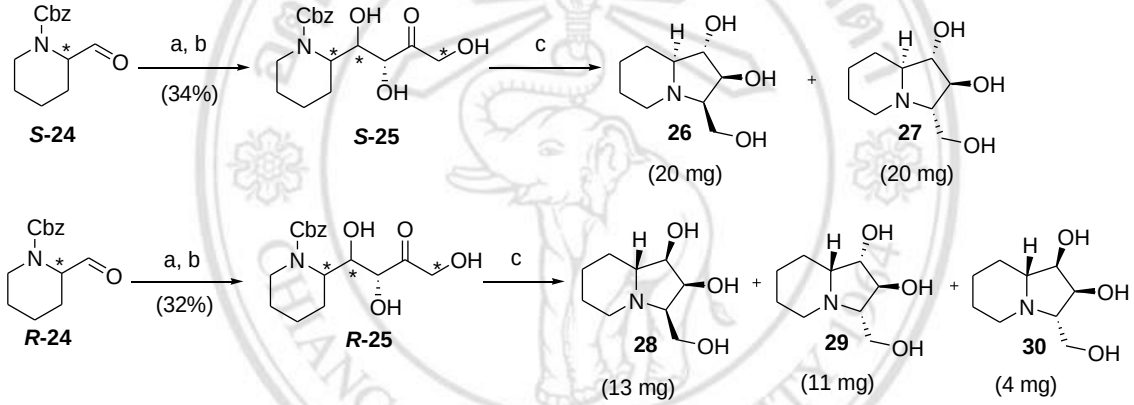
The indolizidine [**23**] was tested against a panel of glycosidases. The results are shown in Table 2.2.

Table2.2. The glycosidase inhibition of compounds **23** (Mean % Inhibition at 1000 µM)

Enzymes	Sources	% Inhibition
α-Glucosidase	Yeast	4.7
	Rice	8.7
	Rat intestinal maltase	24.9
β-Glucosidase	Almond	10.7
	Bovine liver	37.8
α-Galactosidase	Coffee beans	19.2
β-Galactosidase	Bovine liver	34.7
α-Mannosidase	Jack beans	6.7
β-Mannosidase	Snail	0
α-L-Fucosidase	Bovine kidney	0.6
β-Glucuronidase	Bovine liver	6.5
	E. coli	47.8
α,α-Trehalase	Porcine kidney	4.4
Amyloglucosidase	*Aspergillus niger*	7.2
α-L-Rhamnosidase	*Penicillium decumbens*	23.0

Compound **23** had weak inhibitory properties against all of the tested glycosidase enzymes except β-mannosidase (from snail) which was not inhibited.

Gómez *et al.* (194) revealed the chemoenzymatic synthesis of novel indolizidine iminocyclitols in 2012. These compounds were chemo-enzymatically synthesized via two-steps, an aldol addition reaction and a reductive amination reaction. Commercial (*S*) or (*R*)-N-Cbz-piperidin-2-carbaldehyde (***S*-24** and ***R*-24**) were the starting aldehydes for the enzymatic aldol addition reaction with dihydroxyacetone phosphate (DHAP) using L-rhamnulose-1-phosphate aldolase (RhuA) as the catalyst. The expected aldol adducts ***S*-25** and ***R*-25** were converted into the indolizidines derivatives by a one pot, two-step reaction which consisted of N-Cbz-deprotection and reductive amination in the presence of Pd/C with H_2. The iminocyclitols were purified by ion exchange chromatography to give compounds **26-30** (Scheme 2.9).



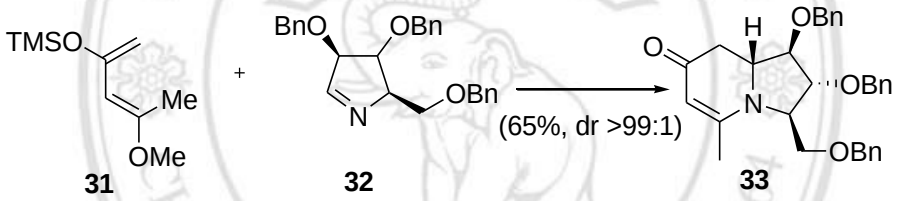
Scheme 2.9. *Reagents and conditions* (a) sodium borate, DHAP, RhuA, DMF; (b) acid phosphatase; (c) Pd/C, EtOH, Water, H_2 (50 psi).

Their structural analysis indicated that the aldol addition of DHAP to ***S*-24,** which was catalyzed by RhuA, gave the *syn* (3*R*,4*S*) configuration of the aldol adduct [***S*-25**], while its enantiomer [***R*-24**] afforded the ***R*-25** adduct as a 2:3 mixture of the *syn* (3*R*,4*S*) and *anti* (3*R*,4*R*) diastereomers, respectively. Reductive amination of ***R*-25** gave the corresponding indolizidines as a 6:1 mixture of *syn* (1*R*,3*R* or 1*S*,3*S*) and *anti* (1*R*,3*S*) diastereomers, respectively. On the other hand, the reductive amination of ***S*-25** was not diastereoselective giving at 1:1 mixture of the *syn* and *anti* isomers.

The synthesized polyhydroxylated indolizidines [**26-30**] were tested as inhibitors against seven commercial glycosidases and rat intestinal disaccharidases. The results showed that compounds **26-28** and **30** were selective inhibitors of α-L-rhamnosidase

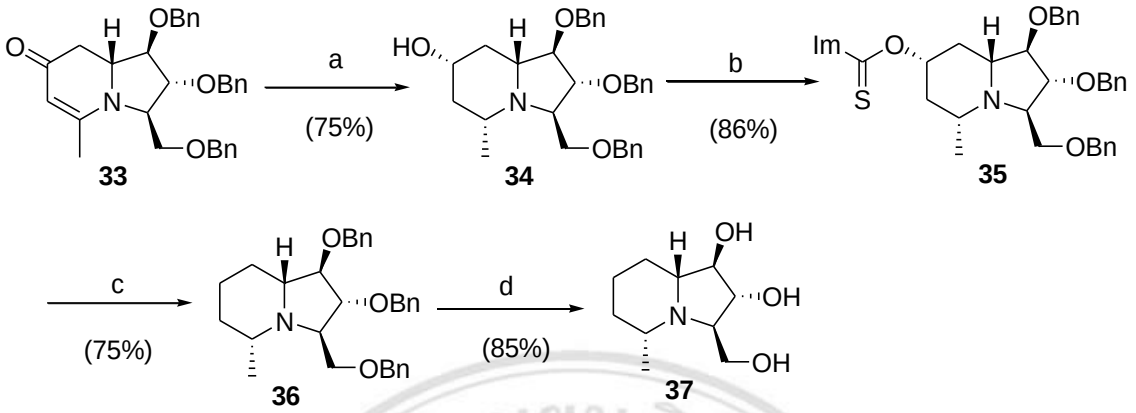
(from *Penicillium decumbens*) with IC_{50} values of 3.3, 113, 35 and 55 μM, respectively, while **28** was found to inhibit α-L-fucosidase (from bovine kidney) and α-D-mannosidase (from jack beans) with IC_{50} values of 82 and 593 μM, respectively. Moreover, product **27** was also found to be a weak inhibitor of α-D-glucosidase (from Baker's yeast) with an IC_{50} value of 237 μM. The inhibitory activities against rat intestinal disaccharide glycosidases showed that compounds **27**, **28** and **30** are selective inhibitors of rat intestinal sucrase with IC_{50} values of 62, 207 and 117 μM, respectively.

In 2012, Shao *et al.* (239) reported the synthesis of (−)-2-*epi*-steviamine [**37**]. Their synthesis started with the key precursor, 5-methylindolizidinone [**33**], which was prepared from the aza- Diels−Alder reaction of the diene [**31**] and the cyclic imine [**32**] (Scheme 2.10).



Scheme 2.10. *Reagents and conditions*: diene (3.0 equiv), imine monomer (1.0 equiv), $Yb(OTf)_3$ (0.5 equiv), anhydrous CH_2Cl_2, −40 °C → 0 °C, 3 h.

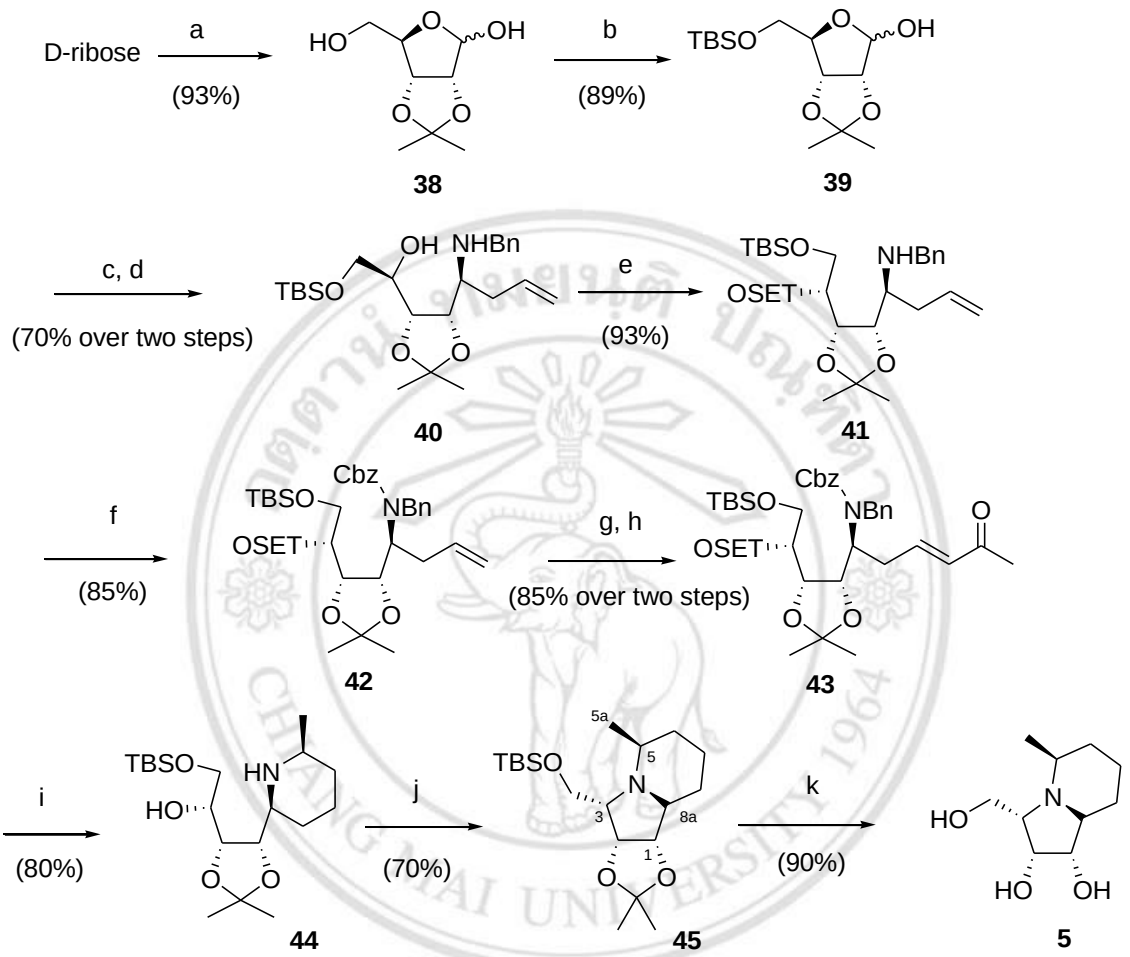
The synthesis of (−)-2-*epi*-steviamine is summarized in Scheme 2.11. The first step involved a fully diastereoselective hydrogenolysis of compound **33** to give the saturated compound **34** as a sole isomer in 75% yield. Compound **34** was then transformed into compound **35** through an esterification with thionocarbonyl-1,1′-diimidazole (TCDI) in refluxing THF, followed by a radical reduction of the compound **35** with tributyltin hydride in the presence of 2,2′- azobisisobutyronitrile (AIBN) to generate the deoxygenated compound [**36**]. Finally, compound **36** was catalytically hydrogenated in an acidic medium to obtain (−)-2-*epi*-steviamine [**37**] as a white solid in a good yield.



Scheme 2.11. *Reagents and conditions*: (a) H_2 (balloon), Pd/C, EtOAc; (b) TCDI, THF, reflux, 6 h; (c) *n*-Bu_3SnH, AIBN, PhMe, reflux; (d) H_2, (30 atm), Pd/C, 6 N HCl, MeOH, 50 °C, 20 h.

An alternative method to synthesize (+)-steviamine was reported by Rajender *et al.* in 2013 (240). They used D-ribose as the starting material which was protected to furnish the 2,3-*O*-Isopropylidene-D-ribose [**38**]. The primary hydroxyl group in compound **38** was protected as its silyl ether using TBDMSCl, imidazole to give compound **39**. The reaction of compound **39** with benzylamine gave ribosylamine and then the crude ribosylamine was treated with allylbromide and zinc generated the amino alcohol [**40**] as a single isomer in 70% yield over the two steps. The secondary hydroxyl group of compound **40** was protected as its triethylsilyl ether to give compound **41**. The amino group in compound **41** was transformed into a carbamate with CbzCl (Cbz = benzyloxycarbonyl) to give the N(Bn)Cbz derivative [**42**] in 85% yield. The ozonolysis of the terminal olefin in [**42**] gave an aldehyde which was subjected to a Wittig olefination reaction by treatment with $Ph_3P{=}CHCOCH_3$ in toluene at reflux to give the (*E*) isomer of the α,β-unsaturated ketone [**43**]. In the next step, a one-pot stereoselective intramolecular reductive amination and selective deprotection of the triethylsilyl (TES) group using 10% Pd/C and ammonium formate in methanol gave the piperidine derivative [**44**]. The secondary alcohol in compound **44** was reacted with MsCl and pyridine to give the corresponding the mesylated compound, which then underwent an intramolecular S_N2 reaction to afford the indolizidine [**45**]**.** In the last step, compound

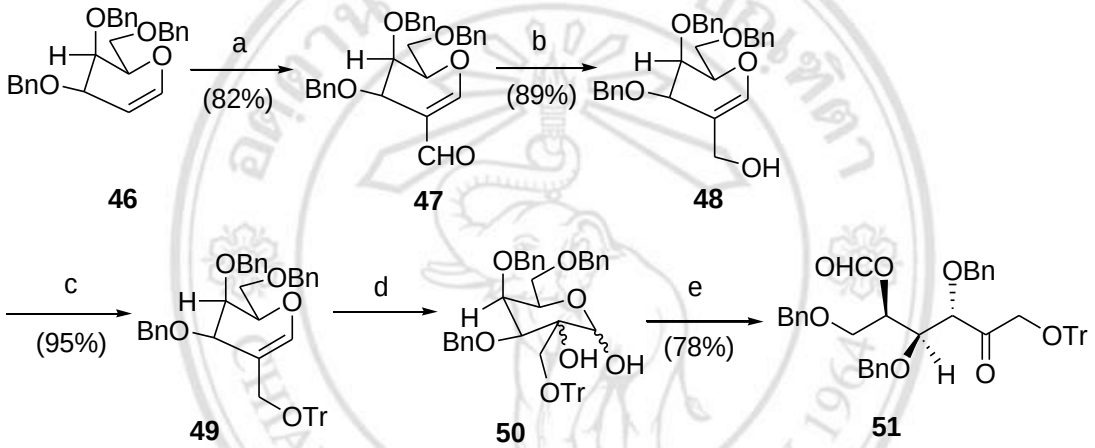
45 was treated with aqueous HCl in ethanol to give (+)-steviamine [**5**] in an excellent yield (90%) (Scheme 2.12).



Scheme 2.12. *Reagents and conditions*: (a) acetone, *c*-H_2SO_4, rt, 2.5 h; (b) TBDMSCl, imidazole, DCM, rt; (c) $BnNH_2$, MeOH, reflux, overnight; (d) allylbromide, Zn, THF, 0 °C to rt, 12 h; (e) TES-Cl, imidazole, cat. DMAP, DCM, 0 °C, 10 min; (f) NaH, CbzCl, THF, 0 °C, 1 h; (g) O_3, DCM, –78 °C and then Me_2S, 0 °C, 2 h; (h) $Ph_3P{=}CHCOCH_3$, toluene, reflux; (i) $HCOONH_4$, 10% Pd/C, MeOH, reflux, 3 h; (j) pyridine, MsCl, rt, 3 h; (k) HCl (6M), MeOH, r.t., 12 h**.**

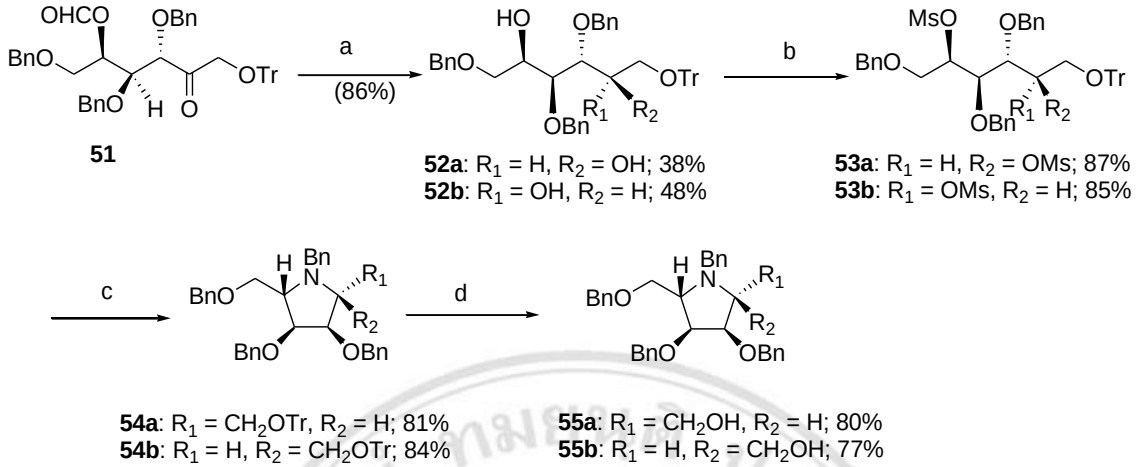
More recently (August, 2013), Ansari *et al.* (241) developed a strategy to synthesize steviamine analogues from C-2 formyl glycals. Synthesis of the monocyclic azasugars started from 3,4,6-tri-*O*-benzyl-D-galactal [**46**] which was converted to the C-2 formyl galactal [**47**] through the Vilsmeier−Haack reaction. Compound **47** was

reduced using sodium borohydride in methanol to generate the allylic alcohol [**48**]. The primary alcohol of **48** was reacted with trityl chloride and triethylamine to give the trityl ether [**49**] in 95% yield (Scheme 2.13). The dihydroxylation of the olefin moiety was carried out using OsO_4 and NMO, furnishing a mixture of diols [**50**]. The crude mixture was subjected to oxidative cleavage using sodium metaperiodate in CH_3CN/H_2O (4:1) at room temperature. The reaction proceeded to completion in a facile manner, when sodium metaperiodate was added in portions over 2 h, followed by vigorous stirring for 3 h at room temperature to obtain compound **51** in 78% yield (over 2 steps).



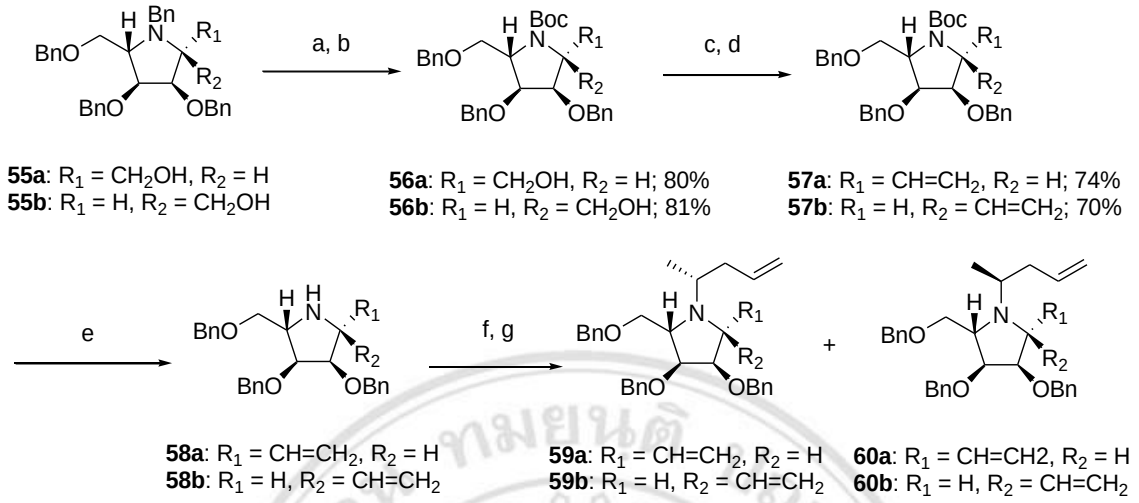
Scheme 2.13. *Reagents and conditions*: (a) $POCl_3$, DMF, 0 °C to rt, overnight; (b) $NaBH_4$, MeOH, 0 °C to rt, 15 min; (c) TrCl, Et_3N, DMAP, CH_2Cl_2, rt, 3 h; (d) OsO_4, NMO, acetone/H_2O/^{t}BuOH, rt, overnight; (e) $NaIO_4$, $NaHCO_3$, CH_3CN/H_2O (4:1), rt, 5 h.

Reduction of **51** using $NaBH_4$ in methanol gave a 1.3:1 mixture of the diols **52a** and **52b** (Scheme 2.14). These diols were separated with a column chromatography and then converted to compounds **53a** and **53b** using mesyl chloride in the presence of triethylamine and a catalytic amount of DMAP at 0 °C. The dimesylates were treated with benzylamine at around 140 °C for double nucleophilic displacements leading to the pyrrolidines **54a** and **54b**, respectively. The trityl protecting group was removed using 3 equivalent of trifluoroacetic acid in dichloromethane at 0 °C giving the alcohols **55a** and **55b**.



Scheme 2.14. *Reagents and conditions*: (a) $NaBH_4$, MeOH, 0 °C to rt, 1 h; (b) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 1h; (c) $BnNH_2$, 140 °C, 6 h; (d) CF_3CO_2H, CH_2Cl_2, 0 °C, 1 h.

Compounds **55a** and **55b** were converted to their *N*-Boc protected amines in order to reduce the nucleophilicity of the amine group (Scheme 2.15). The benzyl group on the amine was removed with $Pd(OH)_2/C$ and 1 atm H_2 in 1 h without affecting any of the benzyl ethers. The crude amine was subsequently protected as its *tert*-butylcarbamate using Boc_2O in the presence of Na_2CO_3 in EtOAc to obtain **56a** and **56b**. Then, the oxidization of the free primary alcohol was carried out using a CrO_3−Py−Ac_2O reagent system and the resulting aldehyde was treated under Wittig olefination reaction conditions using methyl triphenylphosphonium bromide and KO^tBu to generate the alkenes **57a** and **57b**. The *N*-Boc group was cleaved using trifluoroacetic acid in dichloromethane at room temperature over 8 h. The free amine was used for an aza-Michael reaction with crotonaldehyde in the presence of zinc/NH_4Cl. This reaction gave the aldehydes, which were unstable and immediately converted to dienes **59a**/**60a** and **59b**/**60b** using the Wittig reaction.



55a: $R_1 = CH_2OH$, $R_2 = H$
55b: $R_1 = H$, $R_2 = CH_2OH$

56a: $R_1 = CH_2OH$, $R_2 = H$; 80%
56b: $R_1 = H$, $R_2 = CH_2OH$; 81%

57a: $R_1 = CH{=}CH_2$, $R_2 = H$; 74%
57b: $R_1 = H$, $R_2 = CH{=}CH_2$; 70%

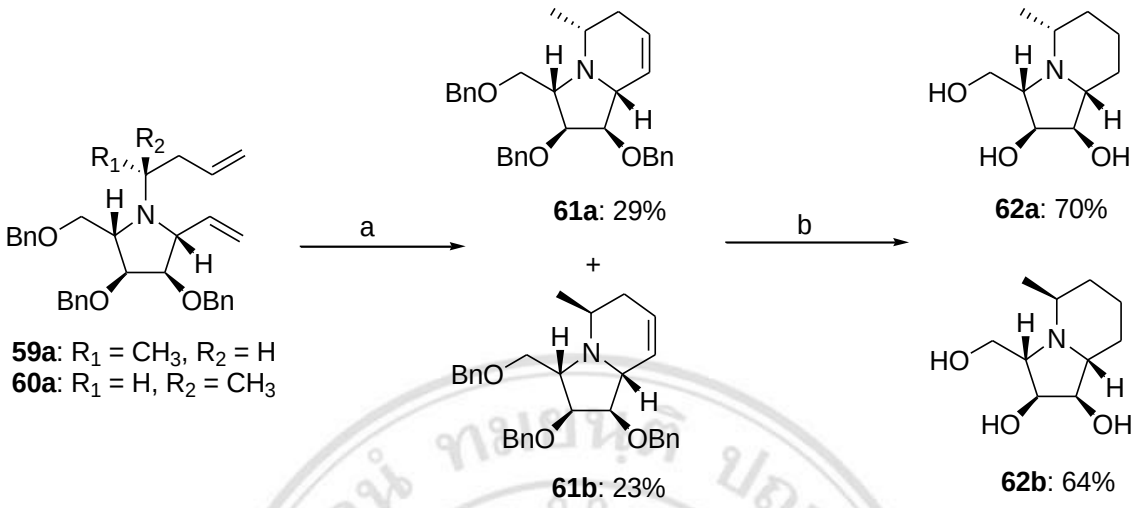
58a: $R_1 = CH{=}CH_2$, $R_2 = H$
58b: $R_1 = H$, $R_2 = CH{=}CH_2$

59a: $R_1 = CH{=}CH_2$, $R_2 = H$
59b: $R_1 = H$, $R_2 = CH{=}CH_2$

60a: $R_1 = CH{=}CH2$, $R_2 = H$
60b: $R_1 = H$, $R_2 = CH{=}CH_2$

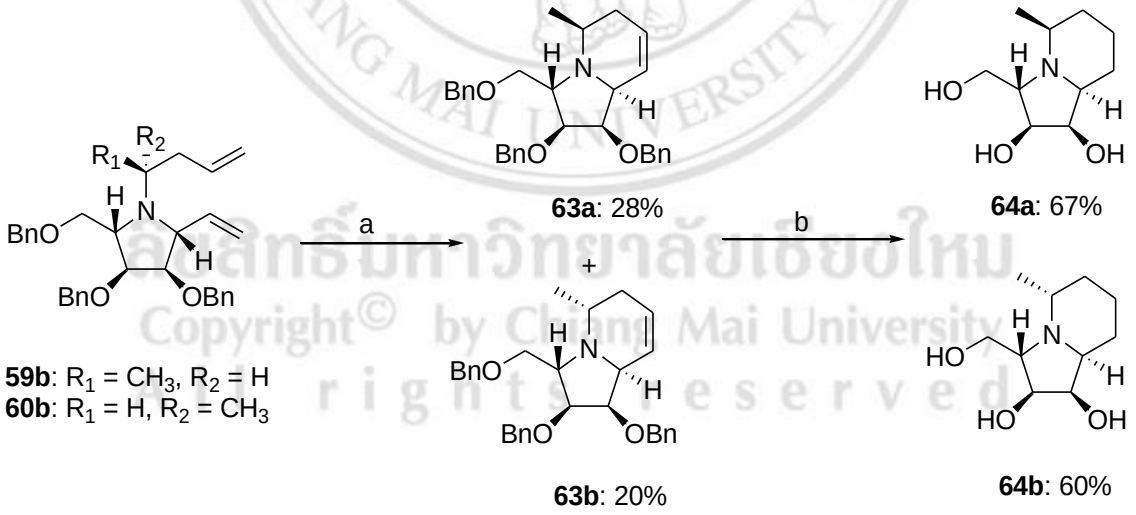
Scheme 2.15. *Reagents and conditions*: (a) $Pd(OH)_2/C$, H_2 (1 atm), MeOH, rt, 1 h; (b) Boc_2O, Na_2CO_3, EtOAc, rt ,3 h; (c) CrO_3, py, Ac_2O, CH_2Cl_2, 0 °C, 30 min; (d) $Ph_3P^+CH_3Br^-$, KO^tBu, THF, 0 °C to rt, 6 h; (e) CF_3CO_2H, CH_2Cl_2, 0 °C to rt, 8 h; (f) Crotonaldehyde, Zn/NH_4Cl, THF/H_2O (5:1), rt, 1.5 h; (g) $Ph_3P^+CH_3Br^-$, KO^tBu, THF, 0 °C to rt, 3 h.

The dienes [**59a** and **60a**] were inseparable by chromatography at this stage, and the mixture was exposed to the ring-closing metathesis reaction with 6 mol % of Grubbs' second generation catalyst in the presence of 2 equivalent of *p*-TsOH in refluxing toluene to afford compounds **61a** and **61b** in a 1.2:1 ratio and in a total yield of 52% over 4 steps (Scheme 2.16). These cyclized products were easily to separate by column chromatography. Each isomer **61a** and **61b** was subjected to one-pot double bond reduction and deprotection of benzyl groups with $Pd(OH)_2/C$ in 10% HCl/MeOH under 1 atm H_2 for 3 days to obtain the steviamine analogues **62a** and **62b** in 70 and 64% yields, respectively.



Scheme 2.16. *Reagents and conditions*: (a) Grubbs'II (6 mol%), *p*-TsOH, toluene, 110 °C, 10 h; (b) $Pd(OH)_2/C$, H_2 (1 atm), 10% HCl in MeOH, rt, 3 d.

The other mixture of dienes **59b** and **60b** underwent the ring-closing metathesis reaction (Scheme 2.17) to give the desired products **63a** and **63b** as a 1.4:1 mixture. Finally, steviamine analogues **64a** and **64b** were obtained after hydrogenation of compounds **63a** and **63b** in 67 and 60% yields, respectively.



Scheme 2.17. *Reagents and conditions*: (a) Grubbs' II (6 mol%), *p*-TsOH, toluene, 110 °C, 10-12 h; (b) $Pd(OH)_2/C$, H_2 (1 atm), 10% HCl in MeOH, rt, 3 d.

All synthesized steviamine analogues (**62a**, **62b**, **64a** and **64b**) were tested against six commercially available glycosidase enzymes. Compounds **62a** and **64a** were found to

be good inhibitors of β-mannosidase (from *Helix pomatia*) with IC_{50} values of 45.6 and 45.9 μM, respectively. The steviamine analogues **62a** and **62b** inhibited α-mannosidase (from Jack beans) with IC_{50} values of 103.3 and 85.8 μM, respectively. All of the steviamine analogues showed weak inhibitory activities against β-galactosidase (from bovine liver) with IC_{50} values of 276.5, 107.8, 812.9 and 412.6 μM, respectively. β-Glucosidase (from almonds) was not inhibited by any of the analogues. All analogues [**62a**, **62b** and **64b**] except **64a** showed preferable affinity toward α-glucosidase (from Baker's yeast) with IC_{50} values of 269.2, 416.3 and 386.7 μM, respectively which corresponded to the results of the α-galactosidase (from coffee beans) inhibition of **62a**, **62b** and **64b**. The IC_{50} values were 116.7, 105.2 and 218.0 μM, respectively.